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In this issue, we present you with 15 articles including 1 review, 4 case reports and 10 original pieces of research from different disciplines. The review published in this issue is about congenital tooth agenesis and its early diagnosis. The aim of this study is to draw attention to the pediatric dentist-pediatrician cooperation in the early diagnosis of congenital tooth agenesis. In one of the research articles in this issue, the authors present an evaluation of syringe feeding compared to bottle feeding for the transition from gavage feeding to oral feeding in preterm infants and they recommend syringe feeding as a transitional method prior to breastfeeding for preterm infants during hospitalization. This issue, with its articles published, covers several scientific areas in pediatrics such as neonatology, pediatric neurology, pediatric oncology, pediatric metabolism, pediatric gastroenterology, pediatric cardiology, pediatric surgery, pediatric infection and pediatric dentistry. This broad range makes our journal special. The impact factor of "The Journal of Pediatric Research" is growing and it gives us hope to enter greater scientific areas and new international indexes.

I would like to acknowledge the members of our editorial board reviewers, authors and Galenos Publishing House for preparing the second issue of 2019. We look forward to your scientific contributions in our future issues. Best wishes
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Sincerely yours,

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Review

Pediatric Dentist-Pediatrician Cooperation in Early Diagnosis of Congenital Tooth Agenesis

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ABSTRACT

Early diagnosis of congenital tooth agenesis increases the quality of treatment options and the final success of the treatment. However, in some clinical conditions, overlooking this diagnosis causes late diagnosis of these anomalies. Late diagnosis reduces the number of appropriate treatments, increases the cost of treatment and may lead to possible complications. In the diagnosis of congenital tooth agenesis, findings during the routine medical examination of the pediatric patient are beneficial. At this point, as well as pediatric dentists, pediatricians also play a great role in early diagnosis of these anomalies. The aim of this study is to draw attention to pediatric dentist-pediatrician cooperation in the early diagnosis of congenital tooth agenesis.

Keywords: Pediatric dentist, pediatrician, early diagnosis, congenital tooth agenesis

Introduction

The term "congenital tooth agenesis" expresses the inherent absence of one or more teeth in primary or permanent dentition (1,2). This situation is more specifically defined by the terms hypodontia, oligodontia and anodontia. Excluding third molars, congenital agenesis of 1-6 teeth is defined by hypodontia, 6 or more by oligodontia and anodontia is used in defining agenesis of all teeth (1,3). Early diagnosis of congenital tooth agenesis ensures optional treatments and affects the quality and success of treatment positively (4-7). However, in situations where the associated cases are not under regular dental control, or where there is no clinical complaint related to the persistent molar tooth in the region with congenital agenesis, this diagnosis may be overlooked (8). In addition, the fact that congenital tooth agenesis is associated with some syndromes, gene mutations and familial predisposition makes it more likely that this deficiency can be diagnosed by medical doctors. Identification of conditions that can point to congenital tooth agenesis in routine check-up by pediatricians of child patients and directing them to the pediatric dentistry clinic makes it possible to carry out the diagnosis in the early period so that the resultant success and quality of the treatment can be increased. This review aims to summarize the familial, syndromic and non-syndromic conditions associated with congenital tooth agenesis and to highlight the early diagnosis, treatment options and the success of treatment obtained by pediatric dentist-pediatrician collaboration.

Prevalence

General Hypodontia Prevalence Among the Population

Congenital agenesis of permanent teeth is the most common dental anomaly (2,9,10). The incidence of this

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anomaly in permanent teeth varies among countries and societies. The incidence of hypodontia being 0.3% in the Israeli population (11) and 26.1% in Thailand's population (12) clearly indicates the difference. In the Turkish population, the prevalence of hypodontia ranges from 2.63% to 8.5% (13-18). However, the incidence of hypodontia in the primary teeth is very low, with a prevalence ranging from 0.1% to 2.38% (10,19-22). In the Turkish population, this rate was reported as 0.2% (23).

Dental Differences in Hypodontia Rates

In the literature, it is reported that, when third molars are excluded, agenesis of mandibular second premolars are the most common among permanent teeth (5,7,24-26), this is followed by maxillary lateral incisors, maxillary second premolars and mandibular incisors (24). Race is considered to be significant in the type of tooth which is affected by agenesis. Endo et al. (27) have stated that, in hypodontia cases; agenesis of mandibular lateral incisors may be more frequent among the Japanese population and Asian studies when compared to other races (9,27). Additionally, evaluations performed on records of orthodontic patients have brought to light that agenesis of maxillary lateral incisors are more frequent (15,16,28,29). This is attributed to the higher rate of referral due to aesthetic concerns caused by the lack of anterior teeth and does not reflect the general population (16). Due to the same aesthetic reasons, studies on orthodontic patients seem to have a higher rate of hypodontia in females, whereas in the general population, the difference among genders is not statistically different (15,16,28-31).

Prevalence of Mandibular Second Premolar and Maxillary Lateral Incisor

The prevalence of agenesis of mandibular second premolars, which is the most common, varies between 1.65-4% (28,32-35), and the absence of these teeth comprises 13-44.9% of all hypodontia (28,34,35). Similarly, congenital agenesis of the mandibular second premolar teeth was found to be the most common in the Turkish population (13,14,17,36-39). It was found that prevalence rates vary between 1.59-3.85% and it comprises 17.7-61.3% of all hypodontia (13,17,37,39). Congenital agenesis of maxillary lateral incisors, which is the second most frequent agenesis following mandibular second premolars, have a 0.8-2.5% prevalence ratio (24,33,40-48).

Etiology of Congenital Tooth Agenesis

Congenital tooth agenesis is linked to defects related to evolutionary, environmental or genetic factors (4,9,49). Tooth agenesis are in fact related to a long-term adaptation and evolutionary process and due to this, it presents with a variety of numeric absences (50).

Congenital tooth agenesis is also related with environmental factors; early radiation exposure of the tooth bud, hormonal and metabolic defects, infections such as rubella, maxilla-facial traumas, osteomyelitis, drugs like dioxin or thalidomide may have an effect (9,51-53).

Etiologic factors of tooth agenesis are also evaluated in terms of syndromic and non-syndromic situations and their genetic bases. Shimizu and Maeda (3) have categorized hypodontia as non-syndromic, syndromic and familial according in its etiology and have stated that congenital tooth agenesis is mostly related to non-syndromic conditions. Additionally, researchers have evaluated the syndromic and non-syndromic cases, which are responsible for tooth agenesis, together with the genes.

Congenital tooth agenesis related with non-syndromic situations are likely to arise due to mutations on the genes responsible for tooth formation Shimizu and Maeda (3). (Muscle Segment Homeobox-1) is one of these genes, which is known for its important role during the tooth formation period (54), and [(Paired Box-9 (PAX-9)] is the gene involved during initialization, bud, cap and bell phases of the tooth formation period and a mutation in this gene results in agenesis especially of permanent molars and second premolars (55). (Axin Inhibition Protein-2) is another gene in which its polymorphic variants are related with hypodontia and oligodontia (56). Moreover, the *PAX* gene family is assumed to be responsible for cellular signalization of cancer cell formation and increasing expression in tumor formation level (57).

Diagnosis of Congenital Tooth Agenesis

Early diagnosis is crucial in establishing optimal treatment options for congenital tooth agenesis, ensuring treatment with the highest success that can be achieved, applying the most minimally invasive treatment options for the patient and eliminating the increased treatment costs of late diagnosis (4-8). The most critical point in this regard is the missed diagnosis and both the pediatric dentist and the pediatrician should perform a detailed clinical and radiological examination of the child patient to prevent this. In the mixed dentition period when both primary and permanent teeth simultaneously can be seen in the dental arch, the absence of clinical signs such as decay, pain, swelling, edema and abscesses in the area of the congenitally absent permanent teeth may lead to congenital agenesis being missed if the patient does not consult a clinic due to the absence complaint (8). Additionally, even in regular dental check-up visits, if the persistent primary teeth in the area do not show any clinical symptoms and no complaint is inferred, the pediatric dentist may skip radiographic screening, thus congenital tooth agenesis may not be detected (8). However, if these patients have a syndrome and are under regular pediatric control,

the pediatrician may facilitate the early diagnosis of tooth agenesis, which is linked with the syndrome, to be made by directing the patient to the pediatric dentist. At this point, after a detailed medical history, followed by clinical and radiological examinations, a pediatric dentist can diagnose congenital tooth agenesis and can take advantage of this early diagnosis.

If the patients do not have a syndromic condition, due to the presence of a clinical complaint in persistent primary teeth in the related area, the pediatric dentist will provide an accurate diagnosis via clinical and radiological examination (8). In this case, the pediatric dentist should direct the patient to a pediatrician for the diagnosis of a possible relation of the agenesis to a syndromic or non-syndromic condition. Thus, both the pediatrician and the pediatric dentist should assist each other in the early diagnosis of both syndromic and non-syndromic conditions, and congenital tooth agenesis associated with these conditions, in a continuous collaboration. If the shedding time of primary teeth has come, the existence of a primary tooth in the dental arch, during transition to permanent dentition and the permanent dentition period, is a clinical sign for congenital agenesis of the permanent tooth in that region (8,58). In a primary tooth retention case like this, a pediatric dentist can diagnose the congenital tooth agenesis by clinical and radiographic examination. This, however, is accompanied by the disadvantage of limited treatment options due to late diagnosis of congenital tooth agenesis (58). One of the most important precautions that can be taken in this case is to make a pedodontic and pediatric evaluation of the younger children in the family of the patient based on the fact that congenital tooth agenesis may be a familial transition, thus, an early diagnosis may be advantageous in these other individuals. Considering the regions where congenital tooth agenesis is most frequently observed, patients applying to dental clinics due to aesthetic concerns are more likely to be diagnosed with permanent lateral tooth agenesis in a persistent maxillary primary lateral incisor case when compared to persistent mandibular primary second molar (8). Thus, both pediatric dentists and pediatricians should carry out a more attentive examination as diagnosis of permanent mandibular second premolar may be overlooked. Considering the regions where congenital tooth agenesis are mostly seen, due to root resorption over time, the root of the persistent primary tooth in both mandibular second premolar and maxillary lateral incisor region becomes fused with the bone as the structure of the periodontal ligament is lost, thus, resulting in ankylosis (4,8,59). In the case of ankylosis detected in a radiographic examination when a certain rate is exceeded, these teeth may stay below the occlusion plane and develop an "infraocclusion" as they cannot physiologically act on the jawbone (4,8,59,60). At the end of mixed dentition and more in the permanent dentition period, these findings in persistent primary teeth, detected both clinically and radiographically, should raise concerns about congenital tooth agenesis (8). The pediatric dentist should confirm the diagnosis of tooth agenesis based on clinical and radiological evidence with careful synthesis of all signs related to congenital tooth agenesis, making absolutely necessary analysis in the presence of such a condition. Subsequently, the pediatrician should be contacted to identify the patient's syndromic or non-syndromic conditions or familial transmission characteristics, similar to previous cases.

Treatment Options in Congenital Tooth Agenesis

In cases of congenital tooth agenesis, early diagnosis of the situation regardless of tooth type, allows the treatment process to be carried out most comfortably by increasing the number of treatment options and quality of treatment outcomes, by ensuring that the treatment process is as non-invasive as possible for the patient, and avoiding costly treatments in cases of late diagnosis allows for optional treatments and increases the success of the treatment, ensures the treatment period is as non-invasive as possible for the patient, avoids high-cost treatments in cases of late diagnosis and thus, allows the most comfortable treatment course for the patient. For this reason, findings that indicate congenital tooth agenesis in patients should be carefully evaluated for early diagnosis and interdisciplinary communication should be conducted if necessary (4-8).

Treatment options for congenital agenesis of the permanent maxillary lateral incisor teeth are generally in the form of conservation of the space resulting from congenital agenesis of the lateral tooth or orthodontic closure of this space (61). The preservation and restoration of the space in the region where the permanent maxillary lateral incisor tooth is missing allows for the maintenance of the natural position of the canine in the dental arch, the ideal class 1 molar occlusion and the continuity of the canine protected occlusion (62). This preserved space is later on restored with fixed/removable prosthodontics or implants when the growth of the patient is complete (61,62). Another approach to congenital agenesis of the permanent maxillary lateral incisor is the orthodontic closure of the mentioned tooth space. This approach is more preferable in cases with class 2 malocclusion, class 1 malocclusion with severe crowding in the maxillary dental arch in which extraction is indicated and in cases of proclined upper incisors (61,62). In this principle, the structure of the alveolar bone is preserved. Later on, the morphology of the canine brought to the lateral incisor region is transformed into the lateral incisor tooth form by applying composite build-up or porcelain veneers (61,62).

Diagnosis of mandibular second premolar agenesis, the most common congenital agenesis in the population, in the early stages of the mixed dentition period also provides optional treatments (7). Fines et al. (5) have stated that there are more treatment options for younger patients, however, these options reduce after 9 years of age. In the early stages, patients with congenital mandibular second premolar agenesis have less-invasive treatment options. The first of them is closure of the space by mesial drifting of the permanent first molar via controlled grinding from the mesial and distal sides of the primary second molars or hemisection followed by extraction (4,63,64). In a right orthodontic profile such as hyperdivergent face type, lack of space in the anterior region/contrary arch and a protrusive face profile, extraction can be done without making the existing profile worse (7,65), thus, in the early stages, achieving the best results from the treatment becomes possible. In the hemisection technique, the permanent first molar is directed towards the space obtained after the extraction of first the distal half and then the mesial half of the primary second molar, by total mesial drift. The parallel movement of the permanent first molar is possible only if the root apices are open, thus the ideal time for hemisection is when the patient is 8-9 years old (8,64). By the time the permanent first molar moves into the space left from the extraction of distal half of the primary molar, approximately within 3-4 months, the mesial half is also extracted, and later on this space is fully closed by the permanent first molar, finally making contact with the adjacent first premolar tooth (8). Additionally, hemisection can be applied combined with the controlled stripping of the distal and mesial surface of the tooth to 1 mm. With regards to root resorption, ankylosis and infraocclusion which may occur in persistent primary teeth, in order to both eliminate these pathologies and to obtain a more natural occlusion, applying hemisection may provide a less invasive treatment period for the patient (8). Hence, in cases of progressive root resorption and ankylosis, the possibility of losing the tooth in advance, and in cases of infraocclusion, the necessity of restoration of the occlusion using composites, compomer, overlay, stainless steel crown and ceramic crown will adversely affect the survival of these teeth (8). Additionally, implants and prosthetic applications after the extraction of primary teeth due to these mentioned pathologies will bring about some disadvantages seen in all artificially applied treatments such as application difficulties, possible complications and high-cost, thus, treatment options in the early stages are more advantageous in terms of obtaining long-lasting success when compared to treatments applied at a later stage. This points out the importance of treatment being applied with an early diagnosis on the success of the treatment (8).

In some cases, even at an early stage, the primary tooth needs to be extracted due to poor prognosis. The extracted tooth's space may either be closed with orthodontic treatment or kept for a future prosthetic, dental implant or autotransplantation application (4,5,7,59,66-70). However, if the space is chosen to be kept orthodontically, due to the fact that implants and prosthetic applications are not initiated until the child patient has reached the end of their growth period, this space should be preserved by space-maintainers for future applications (8). However, this space-maintainer application does not provide for the preservation of the 3D structure of the alveolar bone and causes resorption in all surfaces. This situation brings about both high-cost bone-augmentation procedures in future implant and prosthetic applications and complication risks (8). Therefore, late diagnosis of patients results in a more exhausting and complicated treatment period meaning that the prognosis becomes uncertain. In cases where the primary second molars have a good crown-root structure, are functional and have acceptable esthetic properties, they may be kept in use for a long period (6,71,72), in fact, these teeth can be preserved until 20-30 years of age (59,73).

However, because the mesio-distal width of the primary second molars are greater when compared to second premolars, in order not to adversely affect the occlusion and harmony of the posterior teeth, they should be reduced to the size of premolars. Additionally, in the future, this will make the premolar-formed implant applications possible (65,74). In spite of this, in the preserved primary second molars, because of the risk of progressive root resorption, ankylosis and infraocclusion development, in many cases where these pathologies are progressive and aggressive, these teeth may need to be extracted, which shows that pediatric dentist-pediatrician cooperation in early diagnosis and treatment offers more optimal results and lower-cost treatments.

Conclusion

Dentists or pediatric dentists are the first to diagnose congenital tooth agenesis. Determining tooth agenesis in dentition early increases the potential for functional, aesthetic and stable outcomes. However, considering that hypodontia is often associated with a familial, syndromic or non-syndromic condition, the medical conditions related with the situation can also be diagnosed during the routine examinations of pediatricians. At this point, in cases where congenital tooth agenesis is considered, pediatricians should work in cooperation with dentists or pediatric dentists. Moreover, in addition to hundreds of syndromic conditions related with hypodontia, non-syndromic cases should also be investigated in terms of familial history and dental anamnesis should be obtained, and if needed, contact with a pediatric dentist might be helpful in early diagnosis.

Ethics

Peer-review: Externally peer-reviewed.

Authorship Contributions

Design: Ş.S., Data Collection or Processing: A.D., Ş.S., Analysis or Interpretation: A.D., Ş.S., Literature Search: A.D., Writing: A.D., Ş.S.

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Original Article

Evaluation of Syringe Feeding Compared to Bottle Feeding for the Transition from Gavage Feeding to Oral Feeding in Preterm Infants

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ABSTRACT

Aim: Syringe feeding is a good alternative to a nursing supplementer when breastfeeding is not possible.

Materials and Methods: This study was conducted at a level 2 neonatal intensive care unit in the Zekai Tahir Burak Maternity Teaching Hospital in a comparative and descriptive clinical study pattern. The study was carried out with 47 preterm infants in a syringe-fed group (SG) and 56 preterm infants in a bottle-fed group (BG). Primary outcomes were time of transition from gavage feeding to full oral feeding time of transition from tube to breastfeeding, and hospitalization time. Secondary outcomes were body weight at discharge (g), daily body weight gain (g/days) and gastro-intestinal intolerance symptoms during the transition period.

Results: Mean gestational ages were 29.82±2.03 vs 28.18±1.56 weeks (p=0.24) and mean birth weights were 1,150.31±232.29 vs 1,016.87±186.64 g (p=0.72) in the SG and BG groups, respectively. One hundred and three infants receiving gavage feeding with gestational ages ranging from 26 to 32 weeks were evaluated for full oral feeding start time. Syringe-fed preterm infants had a mean of 40.45±19.50 days and bottle-fed infants had a mean of 53.81±16.97 days (p>0.05). The time to transition to breastfeeding (42.54±21.21 days) and time to discharge (54.48±26.92 days) in the SG was significantly shorter compared to the BG (50.45±15.95, 67.21±22.07, respectively) (p<0.05).

Conclusion: We found that preterm infants for whom feeding with a syringe was used as a reinforcement in addition to orogastric feeding switched to full breastfeeding in a shorter time compared to infants who were fed by bottle. From these results, we recommend syringe feeding as a transitional method prior to breastfeeding for preterm infants during hospitalization.

Keywords: Preterm infants, exclusive breastfeeding, feeding method

Introduction

The physiological limitations of preterm infants less than 32 weeks gestational age make oral feeding difficult. Gavage feeding is the most common and preferred method to initiate enteral feeding in preterm infants (1). Prolonged use of gavage feeding is common in preterm infants who have not yet improved suck, swallow and breathing coordination. However, transition from gavage feeding to breastfeeding or bottle feeding is frequently difficult, resulting in prolonged hospital stays. In addition, research indicates that adverse

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outcomes related to long term gavage feeding include oral stimulation hypersensitivity, esophageal inflammation, pharyngeal desensitization, gastroesophageal reflux and vomiting (2,3). Therefore, prolonged gavage feeding must be avoided.

Preterm infants begin sucking feeds when they are mature enough to co-ordinate sucking and swallowing, which occurs at around 32 to 34 weeks of gestation (4). The alternative oral feeding methods before this time include bottle feeding, cup feeding, spoon feeding, syringe feeding, finger feeding, the use of a lactation aid at the breast or slow-paced bottle feeding. It is important to support the mother in understanding the possible benefits and risks associated with the use of an alternative feeding method. The WHO recommends low birth weight (LBW) infants who need to be fed by an alternative oral feeding method should be fed by cup (or palladai, which is a cup with a beak) or spoon (5)."Nipple confusion" is the term commonly applied to a breastfeeding problem hypothesized to result from the mechanical differences between sucking at the breast and sucking on a bottle nipple (6,7). Scientific studies of nipple confusion are lacking, but there are studies indicating that the mechanics of bottle-feeding and breastfeeding differ (7). Bottle-feeding is not recommended for breastfed preterm infants in order to avoid artificial nipples. Unfortunately, preterm infants, even those who are breastfed at hospital discharge, are often weaned soon after. Many studies have been conducted to evaluate the effects of cup feeding during transition on breastfeeding rates of term infants. No data exists related to syringe use regarding its relevance for feeding preterm infants.

The aim of this study was to compare the effects of syringe and bottle feeding on the passing period to oral feeding, time at passing to full breastfeeding, time discharge of newborns, body weight at discharge and daily weight gain in very LBW preterm infants.

Materials and Methods

This study was conducted at a level 2 neonatal intensive care unit (NICU) between January 2013 and January 2015 in the Zekai Tahir Burak Maternity Teaching Hospital in a comparative and descriptive clinical study pattern. The research group was composed of preterm infants who received treatment and clinical care during the study period and who met the study criteria. The study was approved by the Zekai Tahir Burak Women's Health Training and Research Hospital Ethics Committee (approval number: 22/2018) and informed parental consent was obtained for all infants.

Eligibility criteria included body weight less than or equal to 1.500 g, gestational age younger than 32 weeks, growth parameters appropriate for gestational age, tolerating at least 100 kcals/kg/d oragastric tube feeding at the recruitment to the study, and a stable clinical condition. The exclusion criteria included: grades 3 and 4 periventricular hemorrhage (8); clinical instability at the time or during the study, such as the presence of necrotizing enterocolitis (9) sepsis, bronchopulmonary dysplasia (10), or other respiratory or hemodynamic clinical instabilities; Apgar score <5 at the 5th minute; or presence of genetic syndromes, neurological disorders, or congenital malformation of the head and neck, or of the central nervous system. The research was conducted with 103 premature infants of whom 47 were in the syringe-fed group (SG) and 56 in the bottle-fed group (BG). Start trophic and low-volume gavage feeding within 24 hrs of life. The transition from gavage to oral feeding started when the preterm infants reached at least 32 postconceptional weeks and were tolerating at least 100 kcals/kg/d oragastric tube feeding. The infants were started with one oral feeding per day, and the number of daily oral feedings was increased gradually, depending on how infants tolerated each additional feeding. Each infant's feeding goal was established by their NICU medical team, ranging from 140 to 180 mL/kg/d, and was divided into 8 to 12 portions according to the infant's weight. We implimented a schedule that babies weighing ≥1.500 g be fed three times hourly and those weighing <1.500 g twice hourly. Breastfeeding was started when the preterm infant exhibited signs of physiologic stability and evidence of early feeding behaviors such as rooting, strong sucking on the pacifier and hand-to-mouth behaviors.

Syringe Fed Group: These infants were fed first with a syringe in the oral feeding period. The syringe was placed in the middle of the baby's tongue, gently touching the baby's palate to promote sucking of the syringe. Similar to the finger feeding technique, the nurse gently applied pressure with the syringe while stroking the mouth, gums, tongue and palate. Syringe feeding is shown in Figure 1. The nurse

Figure 1. Syringe feeding baby

then held the baby in an upright position and gently gave no more than 3-5 milliliters of milk or formula at a time into the baby's mouth, the milk being between the gum and cheek or on the tongue of the infant. The baby was then allowed to swallow before giving another 3-5 milliliters of milk or formula until the feed was ended. Our priority was to feed infants with breast milk. However, some infants who did not have a sufficient quantity of breastmilk were fed with formula.

Bottle-Fed Group: These infants were first fed with a bottle during oral feeding. The nipple used was NUK® for milk as it limits the milk flow during sucking.

Vital signs (temperature, respiratory rate, pulse, oxygen saturation) were monitored regularly according to NICU medical procedures. The time required for feeding, weight gain, and episodes of gastro-intestinal intolerance symptoms, and daily weight gain and duration of hospitalization were recorded in the patient's file. The NUK nipple selected for premature newborns was chosen for its anatomic shape and because it was developed for premature newborns who weigh less than 1.750 g. It is made of flexible latex with a very small hole, which forces the preterm infants to compress it to extract milk.

Upon discharge of the infant from the hospital, the mother was counseled with regard to breastfeeding. If the mother's milk supply was inadequate, guidelines were provided for complementation with milk formula according to the feeding routine proposed by the NICU.

Primary outcomes were time at transition from gavage feeding to full oral feeding, time at transition from tube to breastfeeding and length of hospital stay. Secondary outcomes were body weight at discharge (g), daily body weight gain (g/days) and gastro-intestinal intolerance symptoms during the transition period.

Statistical Analysis

Data was analyzed using IBM SPSS Statistics 21.0 (SPSS, Inc, Chicago, IL, USA) statistical package program and statistical significance was set at $p<0.05$. A descriptive analysis of the demographic and clinical characteristics of the patients was conducted. Student's t-test for parametric data or Mann-Whitney U test for non-parametric data were used for comparison of variables between the two groups. Chi-square test was used to compare ratios between the two groups.

Results

One hundred and three infants were included in the intention to treat analysis (SG, n=47; BG, n=56). Characteristics of the participants, including both maternal and neonatal characteristics, were balanced between the groups (Table I). Mean gestational ages were 29.82±2.03 vs 28.18±1.56 weeks (p=0.24), and the mean birth weights were 1,150.31±232.29 vs 1,016.87±186.64 g (p=0.72) in the SG and BG groups, respectively. One hundred and three infants with gavage feeding, ranging from 26 to 32 weeks gestational

Table I. Comparison of baseline characteristics of the study population

Parameters	Syringe group, n=47	Bottle group, n=56	p value
Maternal age, mean ± standard deviation, weeks	28.72±5.8	27.62±6,1	0.35
Gestational age, mean ± standard deviation, weeks	29.82±2.03	28.18±1.56	0.24
Birth weight, mean ± standard deviation, g	1150.31±232.29	1016.87±186.64	0.72
Caesarean delivery, n (%)	41 (45.6)	49 (54.4)	0.82
Female, n (%)	24 (47.1)	27 (52.9)	0.84
Antenatal steroids, n (%)	29 (41.4)	41 (58.6)	0.28
Premature rupture of membrane >18 hours, n (%)	13 (68.4)	6 (31.6)	0,05
Apgar score at 1 min, median (minimum-maximum)	6 (3-7)	6 (4-8)	0.25
Apgar score at 5 min, median (minimum-maximum)	8 (5-9)	8 (5-9)	0.16
Small for gestational age, n (%)	8 (44.4)	10 (55.6)	1
Maternal preeclampsia, n (%)	13 (54.2)	11 (45.8)	0.35
Patent ductus arteriosus, n (%)	14 (45.2)	17 (54.8)	1
Necrotizing Enterocolitis, stage ≥2, n (%)	3	-	0.05
Time to full enteral feeds, mean ± standard deviation	16.65±9.09	20.55±12.75	0.39
LNS, (clinically suspected), n (%)	17 (39.5)	26 (60.5)	0.32
LNS, (culture proven), n (%)	10 (55.6)	8 (44.4)	0.43

LNS: Late neonatal sepsis; mean ± standard deviation

age, achieved a full oral feeding start time, which for syringe-fed preterm infants had a mean of 40.45±19.50 days and for bottle-fed infants had a mean of 53.81±16.97 days (p>0.05). The comparison of syringe and bottle groups is presented in Table II. However, there were significant differences between the groups for transition time to full breastfeeding and time to discharge (p<0.05). There were no significant differences in time to reach birth weight, body weight at discharge, body weight gain (g/day) and time at transition from gavage feeding to oral feeding. The time to transition to full breastfeeding (42.54±21.21 days) and time to discharge (54.48±26.92 days) in the SG was significantly shorter than the BG (50.45±15.95; 67.21±22.07, days, respectively) (p<0.05). There were no significant differences in the prevalence of breastfeeding for preterm infants with syringe feeding compared to bottle feeding at six months after discharge (Table II).

Discussion

Avoidance of bottles in healthy breastfeeding term infants is a basic belief for successful breastfeeding, and hospitals that avoid bottles have significantly higher breastfeeding success rates (11). However, the effect of the "no bottles" technique on breastfeeding success in preterm infants is not fully proven. To do so, there are several methods of providing enteral nutrition to premature infants without using a bottle (12,13). The most commonly used method of feeding preterms other than bottles is cup-feeding. As far as we know, there are no reports on a syringe feeding method for preterm infants. The purpose of this study was to compare the effect of bottle and syringe feeding on the timing of initiating oral feeding and time for transition to full breastfeeding. There were significant differences between the groups in time for transition to breastfeeding, time for transition to full breastfeeding and time to discharge. However, there were no significant differences in time to transition from gavage feeding to oral feeding. Aloysius and Hickson (14) showed administration of oral supplements by cup at a time earlier than infants would be able to co-ordinate sucking, swallowing and breathing for bottle-feeding. Additionally, previous studies reported that cup-fed infants demonstrated significantly more mature breast feeding behaviors when compared to bottle-fed infants (13-15). Dalal et al. (16) found rapid improvement in feeding performance with increasing experience and maturity with paladai feeding. Additionally, they suggested paladai or feeding bottles could be equally safe methods of feeding for hospitalized preterm neonates. The Cochrane review found that cup feeding did not result in longer maintenance of breastfeeding beyond discharge (17). Another review suggested that using a cup instead of a bottle increases the extent and duration of breastfeeding in preterm infants (13). Due to these conflicting results with regard to breastfeeding rates, additional work is needed before the tube alone approach is suggested.

A systematic review by Arvedson et al. (18) also showed positive effects of oromotor interventions for improvement in feeding/swallowing physiology variables. In most infants, the coordination between swallowing and breathing develops to a reasonable level to allow full oral feeding by 32-34 weeks post-conceptional age (19). Feeding performance including coordination of swallowing and breathing improves within a few days of starting paladai feeds thereby helping in early transfer of these infants to their mothers (17).

There are several advantages of syringe feeding in our opinion. First, syringe feeding encourages coordinated breathing, sucking and swallowing in preterm infants. Secondly, syringe feeding allows the infant to control the amount and rate of feeding. Third, syringe feeding results in a positive parental involvement so that mothers

Table II. Clinical profile of neonates

Parameters	Syringe group, (n=47)	Bottle group, (n=56)	p value
Full oral started (postnatal age in days), mean ± standard deviation	40.45±19.50	53.81±16.97	0.17
Time for transition to breastfeeding (postnatal age in days), mean ± standard deviation	42.54±21.21	50.45±15.95	0.04
At discharge days (postnatal age), mean ± standard deviation	54.48±26.92	67.21±22.07	0.01
Time to reach birth weight, day, mean ± standard deviation	13±5.57	15.10±5.10	0.05
At discharge weight (g), mean ± standard deviation	2027.29±357.3	2050.23±434.8	0.77
Body weight gain, g/day, mean ± standard deviation	22.11±12.61	20.19±7.20	0.33
Breastfeeding infants, n (%)	46 (46.5)	53 (53.5)	0.38
Gastro-intestinal motility disturbance, n (%)	14 (38.9)	22 (61.1)	0.40
Abdominal distension, n (%)	14 (40)	21 (60)	0.53
Vomiting, n (%)	13 (48.1)	14 (51.9)	0.82

have early positive body/eye contact resulting in the development of a positive parent-neonate attachment. Fifth, syringe feeding avoids nipple confusion caused by bottle-feeding. Sixth, since disposable syringes can be used, it is less risky in terms of infection than other alternative methods for preterm infants. Complication rates have been reported to be lower during the initiation of feeding in preterm infants. Commonly reported complications with syringe feeding include aspiration and oral thrush. There were no complications in the SG during the hospital stay in our study. Bottle feeding has been associated with the development of increased risk of bacterial contamination resulting in diarrhea and infections both in developed and developing countries (16,6,18). There were no complications in the BG during the hospital stay in our study.

Conclusion

Syringe-fed infants had a shorter time for transition from gavage feeding to oral feeding compared to bottle-fed infants and they had a shorter hospital stay. We therefore suggest supporting the Baby Friendly Hospital Initiative recommendations by using syringe feeding and avoiding bottle-feeding when providing supplementation to gavage feeding for preterm infants.

Ethics

Ethics Committee Approval: Zekai Tahir Burak Women's Health Training and Research Hospital Ethics Committee (approval number: 22/2018).

Informed Consent: Informed parental consent was obtained for all infants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: B.S., F.E.C., Ş.S.O., Concept: B.S., Design: B.S., Data Collection or Processing: B.S., M.B., N.O., G.K.Ş., Analysis or Interpretation: F.E.C., Ş.S.O., Literature Search: B.S., M.B., N.O., G.K.Ş., N.U., Writing: B.S.

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Original Article

Can Serial Measurement Enhance the Diagnostic Value of Procalcitonin as a Marker of Gram-negative Bacteremia in Children with Acute Leukemia?

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ABSTRACT

Aim: Despite improvements in diagnosis and treatment, infections are still major cause of morbidity and mortality in children with febrile neutropenia (FN). In these patients, due to inadequate inflammatory response and subtle clinical symptoms, to determine the source of infection can be challenging. Therefore, it is important to distinguish infections from other non-infectious causes, for both to choose appropriate antibiotic and to reduce the redundant antibiotic use.

Materials and Methods: In this retrospective study, we aim to evaluate serial procalcitonin (PCT) levels for predicting bacteremia particularly caused by Gram-negative microorganism.

Results: Among FN episodes caused by Gram-negative microorganism, the median level of second PCT sample obtained between 48 and 72 hours PCT 2 was found to be significantly higher when compared to infections caused by Coagulase-negative *Staphylococcus* or culture-negative confirmed infections, p value was 0.003; however, fever onset PCT 1 and C-reactive protein (CRP) 1 values showed no significant difference (p>0.05). The area under curve values demonstrated by receiver operating characteristic (ROC) analysis for CRP 1, CRP 2, PCT 1, PCT 2 were 0.664, 0.748, 0.504 and 0.842, respectively.

Conclusion: This study showed that initial PCT levels were not significantly correlate with culture-confirmed bacterial infection. Therefore, initial PCT values do not help the clinicians in terms of administering or postponing empirical antibiotics at the time of fever onset. However, third day PCT levels present as a good diagnostic marker due to a higher sensitivity and specificity when comparing them to the initial values. Determination of serial PCT may enhance the diagnostic value of PCT diagnostic marker in FN episodes caused by Gram-negative bacteria with a high sensitivity (87.5%). This study also demonstrated that PCT could be used to rule out bacterial infections particularly caused by Gram-negative bacteria.

Keywords: Procalcitonin, febrile neutropenia, child, Gram-negative bacteremia

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Introduction

Infections are the most prominent cause of morbidity and mortality in children with febrile neutropenia (FN) (1,2). In these patients, due to an inadequate inflammatory response and subtle clinical symptoms, determining the source of infection can be challenging. Fever sometimes presents as the primary and sole manifestation of an infection adding to the confusion. A positive microbiological culture is only found in 7-31% of febrile episodes (3). Therefore, it is important to distinguish infections from other non-infectious causes, both to choose an appropriate antibiotic and to reduce redundant antibiotic use. A focus of recent studies has been a search for predictors of severe infection and bacteremia. Procalcitonin (PCT) is a precursor of the calcitonin hormone and is excreted in the early phase of infections (4,5). There are several studies suggesting PCT as a better and earlier marker than C-reactive protein (CRP) in children with infections (6-9). A recent meta-analysis evaluating 10 pediatric studies has suggested that PCT is a useful biomarker in detecting microbiologically/clinically confirmed infections with a sensitivity and specificity of 68% and 82%, respectively (6). Another pediatric study has also shown that PCT >2 ng/dL was a good predictor of severe infection [likelihood ratio (LHR) of 26 (95% confidence interval (CI) 3.5, 190)] (9).

This study was conducted to investigate the possible use of PCT levels being used for predicting bacteremia particularly those caused by Gram-negative microorganisms; which are respectively more frequent in our center. The second aim of this study was to determine whether serial measurements of PCT could improve diagnostic value.

Materials and Methods

Thirty-three patients with 51 febrile neutropenia episodes who were hospitalized in the Pediatric Hematology Subdivision of Ege University between February 2014 and June 2014 were evaluated. The patients aged between 1 month and 18 years old were receiving a chemotherapy regimen for acute lymphoblastic leukemia (ALL) (n=27) or acute myeloblastic leukemia (AML) (n=6). During this period, 51 FN episodes were observed. The measurements of CRP 1 and PCT 1 were performed at onset of fever (0-24 hour) and re-measurement was performed within 48-72 hour (CRP 2, PCT 2). Blood cultures were obtained at the onset of fever before the initiation of antibiotics. All patients already receiving antibiotic therapy were excluded. Febrile neutropenia was defined according to the IDSA guideline (10). Bacteremia was defined in patients with a positive blood culture for bacteria (peripheral blood or central venous indwelling catheter), with or without shock. Standard practice in our center includes a thorough daily examination of all patients for clinical signs and sources of infections, as well as monitoring for sepsis and septic shock. Initial blood samples are obtained on the first day and the second draw being performed on the third day at the time the reassessment of antibiotic therapy is recommended. Broad spectrum antibiotics (piperacillin-tazobactam+amikacin or meropenem+amikacin) are initiated after performing blood cultures. Additionally, when an infection is suspected, a urine culture and a cerebrospinal fluid culture are obtained. In those patients with hemodynamic instability, skin and soft tissue infections, or signs of catheter-related infection, vancomycin is initiated empirically.

The medical records of 33 patients whose initial PCT and CRP levels were available were evaluated. The CRP and PCT levels of these patients were also checked and recorded if they were drawn on the third day of admission. The demographic characteristics, medical history, maximum degrees of fever on the day of presentation, fever duration and physical examination findings were recorded. Laboratory findings, including complete blood count, CRP, bacterial cultures and fungal cultures were also recorded. This study was approved by the Ethics Board of Ege University (approval number: 13-4.1/12). All of the parents of the patients gave their informed consent prior to their child's inclusion in the study.

Microbiological Testing

The identification of microorganism was made using VITEK MS (bioMérieux, France). This technology uses Matrix assisted laser desorption ionization time of flight mass spectrometry, which is a new technology used for the identification of species based on the protein composition of microbial cells.

Statistical Analysis

Statistical analyses were performed using MedCalc for Windows (version 15.2 MedCalc Software, Belgium) and SPSS for Windows (version 22.0 SPSS Inc., Chicago, IL, USA). Numerical data were expressed as median (25P-75P). Mann-Whitney U test was used for inter-variable analysis. Comparisons were referred to as statistically significant if the p values were <0.05.

Results

This retrospective study consisted of 51 febrile episodes from 33 patients ALL (n=27) and AML (n=6) with a median age of 92.1 months ranging between 13 and 216 months. The initial PCT levels of 51 episodes, and third day PCT levels of 41 episodes were available (Table I). The mean durations of fever and neutropenia were 3.82 (1-19) days and 9.33 (0-56) days, respectively. Five (9.8%) patients developed septic shock with 4 (7.8%) of them requiring intensive care unit (ICU) admission. The majority of FN episodes [13 (25.4%)] were associated with catheter related bloodstream infection (BSI). This was followed by pneumonia: 3 episodes

(5.8%), catheter exit site infection: 1 episode (1.9%) and zona zoster infection: 1 episode (1.9%). From 13 (25.4%) catheter-related BSI episodes, blood cultures were positive for Gram-positive microorganism in 5 episodes and Gram-negative microorganism in 8 episodes. In all culture-confirmed episodes, the median levels of CRP and PCT on the first day, and on the third day were not found to be statistically different with p levels of 0.494, 0.755, 0.326, 0.592, respectively. However, in Gram-negative microorganism caused infections, the median levels of PCT 2 and CRP 2 showed significantly higher levels when compared to infections caused by CoNS or culture-negative confirmed infections, p values were 0.029 and 0.003, respectively. PCT 1, CRP 1 were not statistically different (p>0.05) (Table II).

In the Gram-negative bacteria infected group and the non-infected group, the values of sensitivity, specificity, positive LHR, negative LHR for CRP 1, CRP 2, PCT 1, PCT 2 demonstrated using ROC curves, are given in Table III. The area under the curve (AUC) showed a statistical significance for CRP 2 and PCT 2 levels (p=0.0015 and p<0.0001, respectively). However, CRP 1 and PCT 1 levels showed no significance (p>0.05). The AUC values for CRP 1, CRP 2, PCT 1, and PCT 2 were 0.664, 0.748, 0.504 and 0.842, respectively. This is summarized in Table III. The optimal cut off values, calculated using ROC curve analysis, were found to be 4 mg/dL for CRP1, 1.3 mg/dL for CRP 2, 0.29 µg/L for PCT 1 and 0.17 µ/L for PCT 2.

Table I. Demographic and clinical characteristics of febrile neutropenia episodes

Characteristics of episodes	
Total number of episodes, n (%)	51 (100)
Duration of fever, median (minimum-maximum) (days)	3.82 (1-19)
Duration of neutropenia, median (minimum-maximum) (days)	9.33 (0-56)
Intensive care unit admission, n (%)	4 (7.8)
Septic shock, n (%)	5 (9.8)
Culture-confirmed infections, n (%)	13 (25.4)
Coagulase-negative *Staphylococcus*, n (%)	5 (9.8)
Klebsiella pneumoniae, n (%)	3 (5.8)
Escherichia coli, n (%)	3 (5.8)
Pseudomonas aeruginosa, n (%)	2 (3.9)

Table II. Comparison of CRP 1, CRP 2, PCT 1, PCT 2 values in febrile neutropenic patients infected with Gram-negative bacteria and non-infected

	Gram-negative bacteria infected [n=8 (15.7%)]	**Non-Gram-negative bacteria infected [n=43 (84.3%)]**	**p value**
CRP 1, median (IQR)	2.80 (3.5)	4.40 (5.43)	0.152
CRP 2, median (IQR)	2.60 (1.1)	0.795 (2.53)	0.029
PCT 1, median (IQR)	0.31 (0.41)	0.295 (0.297)	1.000
PCT 2, median (IQR)	0.40 (4.64)	0.155 (0.1)	0.003

CRP 1 (Fever onset): C-reactive protein, PCT 1 (Fever onset): Procalcitonin, CRP 2 (48-72 hour), PCT 2 (48-72 hour), IQR: Interquartile range

Table III. Diagnostic accuracy of PCT and CRP for predicting Gram-negative bacteremia, results from receiver operating curve analysis

	CRP 1 (FO)	**CRP 2 (48-72 hr)**	**PCT 1 (FO)**	**PCT 2 (48-72 hr)**
Cut-off value	4 mg/dL	1.3 mg/dL	0.50 µ/dL	0.17 µg/dL
Sensitivity (%)	87.5	87.5	37.5	85.7
Specificity (%)	47.6	66.7	79.0	69.7
PPV (%)	22.6	38.9	25	37.5
NPV (%)	95.2	95.7	87.2	95.8
Positive LHR (95% CI)	1.67 (1.1-2.5)	2.62 (1.5-4.5)	1.79 (0.6-5.2)	2.83 (1.6-5.2)
Negative LHR (95% CI)	0.26 (0.04-1.7)	0.19 (0.03-1.2)	0.79 (0.5-1.4)	0.20 (0.03-1.3)
AUC (95% CI)	0.664 (0.516-0.791)	0.748 (0.588-0.870)	0.504 (0.361-0.647)	0.842 (0.692-0.938)
SE	0.122	0.0763	0.119	0.0705
p value	0.1793	0.0011	0.9736	<0.0001

PCT: Procalcitonin, CRP: C-reactive protein, FO: Fever onset, PPV: Positive predictive value, NPV: Negative predictive value, LHR: Likelihood ratio, CI: Confidence interval, AUC: Area under curve, SE: Standard error

Discussion

Children with chemotherapy induced neutropenia may develop infections which rapidly progress to sepsis. Fever can be an initial and the sole manifestation of these infections. Therefore, patients presenting with fever require the initiation of broad-spectrum antibiotics immediately. Using reliable bacterial infection markers can allow health care providers to make an accurate clinical decision regarding antibiotic use and additionally the unnecessary use of antibiotics can be avoided. PCT has been shown to be a reliable marker for distinguishing from other non-infectious causes in patients with FN (7).

CRP and PCT are the biomarkers most commonly preferred in clinical use for predicting bacteremia. In this retrospective study, PCT levels in the second draw on the third day were found to have better diagnostic accuracy than initial values; particularly in detecting FN episodes caused by Gram-negative microorganisms. Gram-negative bacterial infections were satistically significantly associated with higher levels of CRP and PCT on the 3rd day of FN. Both CRP and PCT levels were found to be valuable markers due to their high negative predictive value (NPVs). However, the positive predictive value were found to be low. In a recent study comparing PCT levels in pediatric patients with FN against healthy controls, PCT levels of patients with FN were significantly higher than the controls' (p=0.001). However, the culture-confirmed infections and fever of unknown origin were not compared (11). The malign process itself and also results such as mucositis and graft versus host disease have been shown to stimulate chemokines and to be associated with increased inflammatory markers including CRP, erythrocyte sedimentation rate and leukocyte count. Therefore, investigations focused on PCT as a promising diagnostic marker. A recent meta-analysis evaluated 3.420 FN episodes and the lowest area under curve was found in immunocompromised patients when compared with non-immunocompromised patients and ICU patients (4). Demirkaya et al. (12) evaluated 50 FN episodes of 37 cancer patients and found that PCT levels were seen to be higher in patients with sepsis than those clinically and microbiologically documented infections at admission and on day 3 and day 7. In our study, PCT did not show a significant difference at day of fever onset while its diagnostic value had improved on day 3.

A prospective cohort study by Hemming et al. (9), which included 27 patients with 48 FN episodes, demonstrated that PCT >2g/dL was strongly associated with an increased risk of severe infection [LHR of 26 (95% CI: 3.5, 190). Several previous reports evaluating the diagnostic value of PCT have reported sensitivity and specificity ranging between 93%-96.5% and 70.6%-97%, respectively, in children with fever induced due to chemotherapy (13-15). In contrast, another large prospective cohort evaluated 194 consecutive FN episodes and found that fever onset median PCT levels did not differ between infections and fevers of unexplained origin. However, the diagnostic value did increase on the second day of fever (56% sensitivity, 90% specificity) which is similar to our findings (16). Stoma et al. (17) suggested that PCT is a good diagnostic marker with a 62% sensitivity and an 88% specificity in adult patients with Gram-negative BSI following hematopoietic stem cell transplant. In our study, at fever onset, PCT levels showed a lower specificity (51.1%) in predicting BSI caused by Gram-negative microorganisms. The lower specificity could be attributed to the low ratio of documented infections. On the other hand, higher PCT levels were significantly associated with Gram-negative bacteremia (p=0.018) on the third day. Fleischhack et al. (18) determined that the PCT levels were significantly higher in children with febrile neutropenia caused by a Gram-negative bacteria. Similarly, Reitman et al. (19) reported higher levels of PCT in febrile neutropenic children infected with Gram-negative bacteria than those infected with Gram-positive bacteria. In their study, PCT levels on admission showed a sensitivity of 50% and a specificity of 79%. However, a serial analysis of PCT showed a sensitivity of 78% and a specificity of 76% with an NPV of 96%. They also suggested PCT as a good biomarker for ruling out bacteremia due to high NPV. In this study, we also found third day PCT levels showed an NPV of 95.8% which is similar to previous reports.

The limitations of this study included a relatively low number of FN episodes and its retrospective design.

Conclusion

This study shows that initial PCT levels were not significantly corelated with culture-confirmed bacterial infection. Therefore, initial PCT values do not help clinicians in terms of administering or postponing empirical antibiotics at the time of fever onset. However, third day PCT levels are a good diagnostic marker due to a higher sensitivity and specificity when compared to initial values. This study also demonstrated that PCT could be used to rule out bacterial infections particularly those caused by Gram-negative bacteria. Due to a low rate of culture-confirmed infections in children with chemotherapy-induced neutropenia, monitoring PCT may provide a platform by which antibiotic therapy can be more accurately managed.

Ethics

Ethics Committee Approval: This study was approved by Ethics Board of Ege University (approval number: 13-4.1/12).

Informed Consent: All of the parents of the patients gave their informed consent prior to their child's inclusion in the study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: Z.Ş.B., Design: Z.Ş.B., Data Collection or Processing: Z.Ş.B., G.E., Analysis or Interpretation: Z.Ş.B., Literature Search: Z.Ş.B., Writing: Z.Ş.B., D.Y.K.

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Original Article

Can Temporomandibular Joint Disorders Be Diagnosed Beforehand by Assessment of Postural Irregularities?

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ABSTRACT

Aim: In many studies, the relationship between postural status and temporomandibular disorders (TMD) have been investigated, however there is no consensus on this regard. The aim of this study was to investigate the relationship between postural irregularities forward head posture (FHP) and different shoulder levels (DSL) and TMD prevalence in different dentition stages.

Materials and Methods: This study, which included children between 4 and 14 years of age attending public schools in Ankara, was planned as a cross-sectional study. Temporomandibular joint (TMJ) was examined intra- and extra-orally. After the examination, the relationship between TMD and body posture (FHP and DSL) was investigated. For statistical analysis, chi-square test and Fisher's exact test were used with significance level of $p<0.05$.

Results: FHP and DSL were statistically related to TMD ($p<0.05$). In primary dentition, there was no significant relation between FHP and TMD, however, DSL were found to be significantly related to TMD ($p<0.05$). In mixed dentition, both of these parameters were statistically related to TMD ($p<0.05$). However, in the permanent dentition, there was no relationship between body posture and TMD.

Conclusion: Since there is a strong correlation between postural irregularities and TMD especially in the mixed dentition stage, TMJ and postural status of pediatric patients should be examined as early as possible in the stages of the life. In this regard, the awareness of pediatricians and pediatric dentists to this matter needs to be improved.

Keywords: TMJ, TMD, head posture, body posture

Introduction

The temporomandibular joint (TMJ) is an ellipsoid variety of the synovial joints forming a bicondylar articulation. TMJ includes a disk, fibrous capsule, synovial membrane and ligaments (1). Temporomandibuler disorders (TMD) are defined as neuromuscular and musculoskeletal problems characterized by TMJ, masticatory muscles and clinical findings associated with the related structures (2). TMD are known as functional irregularities of the general masticatory system, TMD associated disk displacements and degenerative and inflammatory diseases of these structures (3,4).

TMD occurs with multiple aetiological factors (5) and common causes are parafunctional habits such as macrotrauma, bruxism and clenching, skeletal and occlusal disorders, psychosocial factors and systemic factors (6). The prevalence of TMD is highly variable due to differences in populations studied in children and adolescents (6). In general, clinical symptoms associated

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with TMD are less common in the primary dentition period than in the mixed and permanent dentition periods (7).

It is known that TMD is positively related to head and body posture (8-13). The body posture is a position associated with muscle activation regulated by the central nervous system (14). The biomechanical organization of the body posture is regulated by the functional integration of the various body structures, and the changes in any biological subunit result in the differentiation and reorganization of the postural control systems (11,13).

The stomatognathic system includes structures which have an important role in postural control such as the lower and upper jaw, dental arches, neurovascular soft tissues and muscle groups related to TMJ (14). The muscular groups of the stomatognathic system belong to the muscle groups of the neck and all units of the muscular chain are related to each other. As a matter of fact, TMJ is a structure which is connected to the neck region via the muscular and ligamental structures and this functional unit is called "cranio-cervico-mandibular" system (14,15). In light of these points, any disorder of the muscular component leads to a reorganization of other subunits (11). Therefore, the relationship between body posture and the incidence of TMD needs to be clearly defined (11,16) in order to provide the optimal treatment for TMD patients.

The aim of this study is to investigate the relation between postural irregularities and TMD prevalence in different dentition stages. The secondary goal of this research is to improve the awareness of pediatric dentists and pediatricians in order to facilitate the early diagnosis of TMD by additional examinations which include postural status.

Materials and Methods

Sample Selection and Ethical Approval

This study, which included children between 4-14 years of age attending public schools in Ankara, was planned as a cross-sectional study including intra-oral and extra-oral examinations. The study protocol was approved by the Ethics Committee of Ankara University (approval number: 150/2). The participants of the study were selected based on a voluntary basis and the parents of the children who participated in the study gave informed written and verbal consent. After detailed information was provided, informed consent forms were signed by the parents. A power analysis was performed to determine the sample size by using the number of children at different dentition groups included in the study (Table I).

Evaluation of the Postural Status

A total of 6 photographs (3 profile, 3 facade) were taken for the postural evaluation of the children. After the participants were positioned on a flat surface, photographs were taken from a distance of 2 meters. Right and left shoulder location levels were evaluated in the facade photographs and the average value of the photographs were recorded as a result. Similarly, in the photographs taken in profile, the location of the shoulder and the ear was evaluated and averages of photographs were

Table I. Sample sizes of different dentition groups

Ages	n
4-6 (Primary dentition)	298
7-11 (Mixed dentition)	669
12-14 (Permanent dentition)	408
Total	1375

Figure 1. Photos taken for posture evaluation and guide lines

recorded. Digital guide lines were used in the evaluation of photographs (Figure 1). The shoulder levels were examined based on a horizontal line and in case of inequality in shoulder location, it was recorded as different shoulder level (DSL). In the photographs taken from the profile, the guide line passing over the shoulders was taken as the criterion and the anterior head position were recorded as forward head posture (FHP).

Examination of Temporomandibular Joint and Diagnose of Temporomandibular Disorders

The examination of TMJ and diagnosis of TMD were performed by observing the bilateral palpation of the skin, muscles and joints and all movements of the mandible. Palpation of the joint was carried out intra-orally and extra-orally. Thus, the presence of tenderness on palpation and irregularities of lower jaw movements were recorded as "tenderness of masticatory muscles and TMJ". The presence of any findings such as deflection, deviation, disk displacement and joint sounds (clicking, popping, and crepitation) in TMJ examination were recorded as TMD. After this detailed examination, mouth opening capacity, maximum mouth opening, laterotrusion, retrusion, protrusion and presence of pain findings were recorded.

Table II. Presence of postural irregularities in different dentition stages

	FHP (%)	DSL (%)
Primary dentition	5.4	5.4
Mixed dentition	5.8	5.5
Permanent dentition	6.4	6.9

FHP: Forward head posture, DSL: Different shoulder levels

Statistical Analysis

The relationship between postural status and TMD in different dentition stages were analyzed using chi-square test and Fisher's exact test with a significance level of $p<0.05$.

Results

The percentage distribution of the postural irregularities based on FHP and DSL in different dentition stages is shown in Table II.

The relationship between postural status and TMD presence in primary, mixed and permanent dentition is given

Table III. Relationship between postural irregularities and presence of TMD in different dentition periods

			TMD						Chi-square test	p value
			Absence		Presence		Total			
			n	%	n	%	n	%		
Primary dentition	FHP	Absence	267	94.68	15	5.32	282	100	Fisher's exact test	0.063
		Presence	13	81.25	3	18.75	16	100		
		Total	280	93.96	18	6.04	298	100		
	DSL	Absence	268	95.04	14	4.96	282	100	Fisher's exact test	0.011*
		Presence	12	75.00	4	25.00	16	100		
		Total	280	93.96	18	6.04	298	100		
Mixed dentition	FHP	Absence	553	87.78	77	12.22	630	100	27.381	0.000*
		Presence	22	56.41	17	43.59	39	100		
		Total	575	85.95	94	14.05	669	100		
	DSL	Absence	552	87.34	80	12.66	632	100	16.325	0.000*
		Presence	23	62.16	14	37.84	37	100		
		Total	575	85.95	94	14.05	669	100		
Permanent dentition	FHP	Absence	317	82.98	65	17.02	382	100	Fisher's exact test	0.107
		Presence	18	69.23	8	30.77	26	100		
		Total	335	82.11	73	17.89	408	100		
	DSL	Absence	316	83.16	64	16.84	380	100	3.180	0.075
		Presence	19	67.86	9	32.14	28	100		
		Total	335	82.11	73	17.89	408	100		

TMD: Temporomandibular disorders, FHP: Forward head posture, DSL: Different shoulder levels, *Statistically significant difference

in Table III. According to the results, there was no relation between TMD and the FHP in primary dentition, while the presence of TMD was found to be statistically significant in DSL ($p<0.05$). Additionally, the presence of TMD was found to be statistically significant ($p<0.05$) in both postural irregularities in mixed dentition. In permanent dentition, both postural irregularities were not statistically related to TMD. Nevertheless, it has been determined that the rate of TMD was higher in the individuals in whom the postural irregularities were observed.

The rate of presence of TMD was 34.6% in the presence of FHP, whereas the rate of TMD in normal head position was 12.1%. The presence of TMD was statistically significantly higher in the presence of FHP ($p<0.05$) (Table IV). Additionally, the incidence of TMD was 33.3% and 12.2% in patients with and without DSL, respectively. The presence of TMD was statistically significantly higher in the presence of DSL ($p<0.05$) (Table IV).

Discussion

TMD are common diseases in children and adolescents being at least as prevalent as for adults (17-20). TMD develop with multiple etiological factors and occur with signs and symptoms affecting the joint related muscular and neuromuscular components (21-23). In the treatment of TMD, in order to provide preventive procedures, it is necessary to investigate factors that may lead to this disease especially during different dentition periods.

Regulation of the postural structure of the body is possible if the changes in the biological subunit reorganize the postural control systems (11,13). The neck muscles that are adjacent to the TMJ region play an important role in providing the balance of the head and stomatognathic muscles. It means that any level of differences in these structures can cause changes throughout the whole complex. The masticatory muscles can be affected by alterations in head posture and vice versa. Thus, any manipulation of the mandibular muscles can lead to changes in head posture (13,24) and changes in the cervical spine structure can also play a role in developing TMD (13).

As the TMJ region is directly adjacent to the cervical and scapular regions, postural changes in the upper neck and head region correlate with TMD (13). Gonzalez and Manns (25) stated that FHP was caused by an extension of the upper cervical spine (C1-C3) and a flexion of the lower cervical spine (C4-C7) called hyperlordosis. The authors also emphasized that hyperextension was observed in the head and upper cervical spinal region in TMD patients. Alarcón et al. (26) suggested that the position of the jaw can affect the muscles in the peripheral region and cause postural adaptations at the spine level. Asymmetric malocclusions such as unilateral cross bite have been reported to be a risk factor for unbalanced muscle activity and postural dysfunction. Similarly, Solow and Sonnesen (27) stated that there is a strong relationship between the cervical lordosis grade and vertical craniofacial morphology, overjet, class 2 and class 3 anomalies. It is thought that TMD and postural irregularities are related and risk factors for each other. In this way, it can be stated that there is a positive relationship between head/body posture and TMD (8,9,11). In the present study, the aim was to investigate the relationship between TMD and head/body posture and this relationship was examined in different dentition periods. The presence of disk displacement, joint sounds, muscular irregularities, movement limitations and pain were accepted as TMD. In postural status evaluation, FHP and DSL were examined.

In order to determine the postural status, several techniques (e.g. surface electromyography, kinesiography, different clinical and instrumental posturographic approaches) have been used over the years (28). Despite some studies on the stomatognathic system and its relationship with posture (29-33), they have restrictive factors to their clinical application because of the absence of normative values for age, sex, weight, height and facial

Table IV. The relationship between postural irregularities and TMD

		TMD						Statistical analysis	
		Absence		Presence		Total			
		n	%	n	%	n	%	Chi-square	p value
FHP	Absence	1137	87.9	157	12.1	1294	100	31.051	0.000*
	Presence	53	65.4	28	34.6	81	100		
	Total	1190	86.5	185	13.5	1375	100		
DSL	Absence	1136	87.8	158	12.2	1294	100	27.423	0.000*
	Presence	54	66.7	27	33.3	81	100		
	Total	1190	86.5	185	13.5	1375	100		

TMD: Temporomandibular disorders, FHP: Forward head posture, DSL: Different shoulder levels, *Statistically significant difference

morphology. For these reasons, this study was carried out in an out-of-clinical setting and simple photographic techniques were used as an evaluation criterion.

In the present study, the presence of TMD was found to be statistically significant ($p<0.05$) in those individuals with FHP and DSL in mixed dentition. In permanent dentition, an association with both postural parameters and TMD was not observed as statistically significant. Similar to the findings of this study, it has been reported that TMD is not frequently observed during primary dentition, while it is increasingly observed during mixed dentition (7). Chaves et al. (34) reported that the alterations in head position were observed in 56% of cases of moderate to severe TMD in individuals in the 10-18 years age group. Cortese et al. (35) stated that FHP was one of the most common postural anomalies at 10-15 years of age, and this was a risk factor for TMD. It seems that there is a need for more standardized studies concerning the relationship between postural irregularities and TMD in different dentition periods in children.

Patients in the mixed dentition period should be carefully assessed for the presence of TMD and postural irregularities, since TMJ related diseases are expected to increase especially after the primary dentition period. It has been reported that TMD stimulate the effects of masticatory muscles on the compensator mechanism and this biomechanical adaptation pulls the shoulders upwards (34). The occurrence of postural disorders is believed to be due to excessive stresses on the cervical muscles depending on the increased activity of the masticatory muscles to compensate for joint disease in TMD individuals (34). Nicolakis et al. (9) reported that training for postural correction in those patients with TMJ disk displacement have had promising results. Consequently, it is not certain that TMD is caused by postural changes or postural disorders are caused by TMD (36). In this respect, both situations should be considered as a potential risk factor for each other. These patients should be diagnosed and treated as early as possible by medical doctors, pediatricians and pediatric dentists using multidisciplinary approaches.

Conclusion

TMD is not only a problem affecting adults but can also be seen in pediatric patients. However, when considering the relationship between postural status and changes and presence of TMD, it is unclear which one is the etiological factor for the other. The disorders mentioned should be diagnosed at the youngest possible age. This approach will be beneficial in terms of the elimination of both TMD and postural irregularities. Therefore, it is recommended that pediatric dentists and pediatricians should evaluate the findings that may raise suspicions about the diagnosis of TMD and postural irregularities in a multidisciplinary manner.

Ethics

Ethics Committee Approval: The study protocol was approved by the Ethics Committee of Ankara University (approval number: 150/2).

Informed Consent: Informed consent was obtained by the parents.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: C.D., N.Ö., Concept: C.D., A.D., N.Ö., Design: C.D., A.D., N.Ö., Data Collecting or Processing: C.D., N.Ö., Analysis or Interpretation: C.D., A.D., N.Ö., Literature Search: C.D., A.D., N.Ö., Writing: A.D., N.Ö.

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Original Article

Visceral Leishmaniasis in Children in Southern Turkey: Evaluation of Clinical and Laboratory Findings and Liposomal Amphotericin B Treatment

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ABSTRACT

Aim: Visceral leishmaniasis (VL) is a systemic infection that spreads hematogenously and affects the reticuloendothelial system by the infection of macrophages. VL occurs commonly in children, and only rarely in adults. VL should be considered in patients with prolonged high fever, hepatosplenomegaly, pancytopenia, weight loss, pallor and hypergammaglobulinemia.

Materials and Methods: In this study, a total of 18 pediatric patients -9 (50%) males and 9 (50%) females- treated for VL at our clinic from January 2004 to July 2014 were analyzed retrospectively. Average time from symptom onset to hospital admission was 64±21 days (range: 30-100 days). The mean age of patients was 88±40 months (range: 36-182 months).

Results: The most common symptom at presentation was fever (88.9%). Other common symptoms were fatigue, chills, weight loss and anorexia. Physical examination revealed splenomegaly and hepatomegaly in all patients. Anemia (92.4%), leukopenia (78.7%) and thrombocytopenia (76.2%) were the most prominent laboratory abnormalities and 82.2% of the patients were pancytopenic on admission. Bone marrow smear was positive for leishmania in 100% of the patients. All patients received treatment with liposomal amphotericin B.

Conclusion: In certain regions, increased humidity rates associated with construction of dams and irrigation canals may lead to changes in the ecological balance and thus cause an increase in the population of disease-spreading vectors. Additionally, recent migration from the middle-eastern region to western parts of the world due to regional civil wars may have contributed to the observed increase in the incidence of various diseases such as VL.

Keywords: Leishmania, visceral leishmaniasis, liposomal Amphotericin B, child, Turkey

Introduction

Leishmaniasis is a form of parasitic disease with zoonotic-anthroponotic features which is caused by protozoa belonging to the kinetoplastida order, which are transmitted by the bite of sand flies (Phlebotomus or Lutzomyia) (1). It is considered as an epidemic disease which manifests with severe systemic effects. The disease may present as cutaneous, mucocutaneous or visceral leishmaniasis (VL). Globally, around 500.000 new VL cases are reported annually with a death toll of 60.000 (2). In this respect, it is accepted as the second most common cause of mortality among all tropical diseases (3). Vector borne diseases are an important public health problem in Turkey, especially in humid regions and regions that have borders

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with neighboring war-ridden countries in which public health-care is on the verge of collapse. Officially, there have been 207 VL cases in Turkey during the last decade. The ongoing refugee crisis and the favorable climate of these regions may be contributing to the spread of vector-borne diseases (4).

VL is a systemic infection in which macrophages are infected through hematogenous spread. In developed western countries, a patient with VL is a rare occurrence and it is seen as an opportunistic infection affecting those with immunosuppression. In the tropical climate zone and Mediterranean basin countries, it occurs endemically (5). The most common etiologic agents include Leishmania (L) donovani, L. infantum and L. chagasi, while L. tropica and L. amazonensis are also known to cause the disease. In Turkey, it is seen endemically in the Southeastern, Mediterranean and Aegean regions. Clinically, it is characterized by a sustained high fever that is resistant to non-specific treatments and which has the following symptoms: pallor, weight loss, hepatosplenomegaly, pancytopenia and hypergammaglobulinemia. Due to these clinical and laboratory features, it resembles malaria, tuberculosis, brucellosis, salmonella infections or malign hematologic diseases (1-3). Thus, it is important to remind clinicians of the characteristics of VL in order to prevent misdiagnoses and provide prompt treatment to patients, especially considering the rarity of VL cases.

Our aim was to review the relevant literature and to investigate the characteristics of pediatric VL, which is occasionally endemic in our region and presents a serious health problem in our country.

Materials and Methods

In this study, 18 pediatric patients diagnosed and treated for VL in our clinic between January 2004 and July 2014 were evaluated retrospectively. Demographic characteristics and the clinical and laboratory findings of all cases were retrospectively assessed from their clinical records.

All patients underwent bone marrow aspiration (BMA) for diagnostic purposes. BMA samples were assessed by the departments of Pediatric Hematology and Pediatric Infectious Diseases. The diagnoses of all patients were confirmed by the demonstration of parasite amastigotes in Giemsa-stained smears prepared from BMA samples. In five patients (27.8%), promastigotes were isolated from cultures in Novy-MacNeal-Nicole (NNN) medium (Figure 1). Additionally, the RK-39 dipstick test was performed with all bone marrow aspirates.

In order to confirm the etiology of non-specific fever and hepatosplenomegaly, all patients were examined with posteroanterior chest X-ray, purified protein derivative test, blood-, urine-, throat- and sputum cultures, thick blood smear, peripheral blood smear, serum Wright agglutination test, Gruber-Widal agglutination test, echocardiography, toxoplasmosis immunoglobulin M (IgM), CMV IgM, EBV VCA-IgM and collagen tissue disorder markers (antinuclear antibody, Anti-DNA, RF) for differential diagnosis of VL.

All patients underwent BMA; the aspiration was repeated in 4 patients with non-specific results to confirm the diagnosis. Three cases were diagnosed by the incubation of promastigotes in NNN media culture. Bone marrow or liver biopsies and spleen aspirations were not required in any of the patients.

All patients were treated with liposomal Amphotericin B (Ambisome) administered 3 mg/kg/day parenterally on days 1-5, 14 and 21 (a total of 7 doses). Post-treatment cure was determined as diminishment of symptoms, normal body temperature, regression of spleen size, normalization of laboratory findings and finally, the absence of leishmania amastigotes on repeat bone marrow aspirates. Because of the retrospective nature of this study, it is outside the scope of the ethics committee, and so we did not apply to ethics committee. The parents of the patients included in this study provided informed consent for the patients data to be used.

Results

The study population included 18 VL patients, 9 of whom were male (50%) and 9 were female (50%). The mean age of the patients was 88±40 (range: 36-182) months. The provinces where the patients resided were Adana (5), Hatay (4), Şanlıurfa (3), Osmaniye (2), Diyarbakır (2), Gaziantep (1) and Kahramanmaraş (1); all of these cities are located in Southern Turkey and have relatively humid climates due to their proximity to the Mediterranean Sea. Eleven patients (61.1%) were living in a rural setting, while 7 (38.9%) were from urban areas. Twelve patients (66.7%) had a history of contact with animals. None of the patients had a previously diagnosed immunosuppressive condition. All patients had

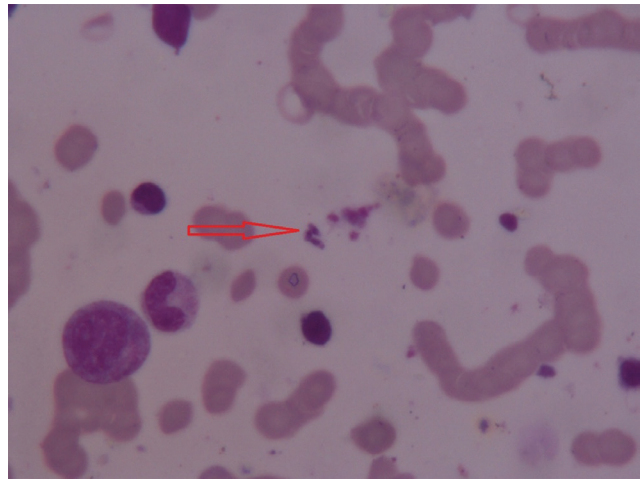

Figure 1. Light-microscopic examination of a stained bone marrow

received inpatient antibiotic treatment in another hospital before admission to our clinic.

The most common complaint on admission was fever (88.9%) (Table I). Other common symptoms were fatigue, chills, weight loss and loss of appetite. Physical examination revealed hepatomegaly and splenomegaly in all patients. Time from onset of symptoms to diagnosis was 64.05±21.37 days (30-100 days). Laboratory test results revealed pancytopenia, hypoalbuminemia and a high erythrocyte sedimentation rate (ESR) (Table II).

Discussion

Leishmaniasis is a zoonotic infection which is endemic in the Mediterranean and Aegean regions of Turkey, while the south-eastern region has seen an increase in the number of cases reported in the last few years. Official reports put the total number of VL cases during the last decade at 207. We hereby report 18 cases with VL that were diagnosed and treated at our center. None of the patients had a recurrence during 6 months of follow up.

Table I. Frequency of symptoms for visceral leishmaniasis on admission

Symptom	No	%
Fever	16	88.9
Fatigue	14	77.8
Chills	14	77.8
Lack of appetite	12	66.7
Pallor	10	55.6
Weight loss	10	55.6
Abdominal distension	6	33.2
Low blood count	4	22.2
Cough	4	22.2
Diarrhea	3	26.7

Table II. Laboratory and physical examination findings of visceral leishmaniasis

Sign	No	%
Anemia	18	100
Thrombocytopenia	18	100
Neutropenia	18	100
Splenomegaly	18	100
Hepatomegaly	18	100
Elevation of liver enzymes	18	100
Increased ESR and CRP	18	100
Pyrexia	15	83.3
Lymphadenopathy	9	50
Hypoalbuminemia	6	33.3
Cachexia	4	22.2
Petechia	2	11.1

ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein

Initial Symptomatology

Sixteen (88.9%) of our patients presented with fever, while fatigue (77.8%) and chills (77.8%) were the second most common symptoms at presentation. Other symptoms in order of reducing frequency were as follows: loss of appetite (66.7%), pallor (55.6%), weight loss (55.6%), abdominal distension (33.2%), low blood count (22.2%), cough (22.2%), and diarrhea (16.7%) (Table I). Similar to our findings, fever has been reported tof be the most frequent symptom among VL patients. Fever frequency has been reported as 94.2% in a study from Iran (6), 100% in the Black Sea Region of Turkey (7), 95% in Greece (8), 95% in France (9), and 98% in China (10). Although the literature on VL is rather unanimous regarding symptom frequency, a few studies which report varying findings exist. For instance, a study by Krepis et al. (11) reported that all (100%) of their patients had pallor; however, in the current study, pallor was observed in only 55.6% of patients, while Miao et al. (10) reported a frequency of 33%. Among our patients, loss of appetite (66.7%) and weight loss (55.6%) were also primary symptoms, while in the study by Krepis et al. (11) loss of appetite and subsequent weight loss were evaluated together, and were observed in just 32.6% of their cases. The same study reported that 16.3% of their cases had vomiting/diarrhea, which is very similar to our findings.

Physical Examination and Laboratory Findings

All patients were found to have hepatosplenomegaly at presentation (100%), while pyrexia was found in 83.3%, lymphadenopathy in 50%, cachexia in 22.2%, and petechiae in 11.1%. In other studies, splenomegaly (86-100%) and hepatomegaly (58.1-98%) are also reported to be the most frequent physical examination findings (6,10-12) (Table II). However, the frequency of hepatomegaly (100%) in the current study group was relatively high compared to studies by Krepis et al. (11) (58.1%), Abdinia et al. (6) (51.3%) and Miao et al. (10) (74%). It is also important to note that Abdinia et al. (6) found that liver involvement was present in all cases of mortality in their patient group. Lymphadenopathy was the third most common finding in our study group with a frequency of 50%, however, Miao et al. (10) reported that it was present in only 33% of their patients. In the current study, bleeding manifestations were observed in the form of petechiae in 2 (11.1%) patients, Krepis et al. (11) reported bleeding manifestations in 7% of their patients (12), while Abdinia et al. (6) reported petechiae and purpura in 11.5% of their study group.

The classic triad of VL is comprised of fever, splenomegaly and pallor (13,14). In the current study, the frequency of fever and splenomegaly conformed to the triad; however, pallor was observed in only 55.6% of patients. This is an irregular finding which may be caused by several of the differences between study populations including race, time until admission to center and previous treatments at other centers.

Laboratory findings in VL comprise varying levels of anemia (normocytic and normochromic), neutropenia, eosinopenia, thrombocytopenia, and an elevation of bilirubin and liver enzymes (15). In the current study, significant anemia, leukopenia, increased ESR and C-reactive protein (CRP) in addition to slight-to-moderate thrombocytopenia were present in all cases. Also, the majority of patients had decreased albumin levels with normal total protein concentration. Similarly, Krepis et al. (11) reported a decrease in the albumin/globulin ratio in 72.1% of their patients.

Anemia, slight-to-moderate liver enzyme elevation, and increased ESR and CRP were present in all (100%) of our patients at presentation, which conforms to the majority of previous studies (1,5,13). However, in a study by Abdinia et al. (6), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) elevation were reported in only 25% and 11% of patients, respectively. This can be seen as a major difference, especially considering that the study in question was performed in a region that neighbors the south-eastern region of Turkey (Northwest of Iran). The majority of patients in our study resided in areas close to the Mediterranean Sea; whereas the Northeast of Iran is close to the Caspian Sea. Therefore, there may have been major differences in the type of Leishmania and/or vectors in these regions. Another cause for this difference may be simply explained by differences in reference ranges for AST and ALT in various studies.

Treatment

Leishmaniasis is traditionally treated with antimonial drugs, namely sodium stibogluconate and meglumine antimoniate (16). Although these are effective and cheap drugs, antimonials may cause severe cardiac and gastrointestinal side-effects (17). Miltefosine is another widely used treatment option with less frequent and less severe side effects compared to antimonials. However, a particular drawback of miltefosine is its lesser efficacy in those with human immunodeficiency virus, which could limit its use in developed countries where VL usually develops as an opportunistic infection in those with immunosuppression (18). Liposomal Amphotericin B is currently considered the most effective and safe drug for the treatment of VL and the only drug with FDA approval (19), even though it is very expensive compared to the aforementioned treatments.

All of our patients received treatment with liposomal Amphotericin B at a parenteral dose of 3 mg/kg applied on days 1 to 5, 14 and 21 (cumulative dose of 21 mg/kg). All patients were cured, no side effects were observed and none of the patients had a relapse during 6 months of follow-up.

The dosage used in the current study was relatively high compared to suggestions in studies from India that describe 89% cure rate with a cumulative dose as low as 3.75 mg/kg and 97% with 15 mg/kg (20). However, studies from other regions report lower cure rates with such doses. For instance, in Brazil, a treatment dose of 2 mg/kg per day for 10 days (total 20 mg/kg) was found to cure 87% of patients, while a total dose ranging between 7-14 mg/kg was successful in only 62% of cases. Various studies from different parts of the world have confirmed that a cumulative dose of 18-21 mg/kg achieves almost complete success in patients with VL (21-23). As such, the current FDA recommendation for the treatment of VL with liposomal amphotericin B is 3 mg/kg on days 1-5, 14, and 21 (cumulative dose: 21 mg/kg) (19). It is also important to note that some studies suggest that a high initial dosage (5-10 mg/kg) is instrumental in obtaining optimal tissue levels as soon as possible (24). This approach may also reduce hospital stay and is reported to be preferred by some pediatricians in Europe (12).

Conclusion

While our patient group showed significant differences in some clinical and laboratory findings compared to previous studies, the 100% cure rate of VL with a total dose of 21 mg/kg is a demonstration of the efficacy of treatment with liposomal Amphotericin B. Recommendations of short- and long-term regimens are still a matter of debate, as both treatment courses have been shown to be effective in various studies. However, it is crucial to evaluate regional differences when considering treatment approach and we suggest following the FDA-approved treatment guideline in the majority of cases; however, severe cases may benefit from an initial high dosage. Further studies with varying dosage evaluations that are prospectively designed should be performed in order to elucidate optimal treatment with liposomal amphotericin B.

Ethics

Ethics Committee Approval: Retrospective study.

Informed Consent: The parents of the patients included in this study provided informed consent for the patients data to be used.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: B.Ş.K., E.K., E.A., İ.Ş., B.A., Concept: B.Ş.K., Ö.Ö., Design: B.Ş.K., Ö.Ö., E.A., Data Collection or Processing: B.Ş.K., Ö.Ö., İ.Ş., Analysis or

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Original Article

Pediatric Liver Transplantation Outcomes for Metabolic and Non-metabolic Diseases in Turkey: A Single Center Experience

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ABSTRACT

Aim: Liver transplantation (LT) is performed for several indications in the pediatric population, including malignancy and acute hepatic failure among others. LT has become an important treatment alternative for metabolic diseases. In most pediatric transplant centers, metabolic liver disease is the second most common indication for LT after biliary atresia. Our studies aim is to compare the post-transplant outcomes of those patients with metabolic and other liver diseases in our transplant program.

Materials and Methods: One hundred eighty-nine patients who underwent LT between 1997 and 2015 due to metabolic diseases and acute or chronic liver failure were included in the study. This study was performed retrospectively.

Results: We enrolled 189 patients in our study. 54% (n=102) male and 46% (n=87) female patients were included in the study. The metabolic disease group included 56 patients and the non-metabolic disease group contained 133 patients. Progressive familial intrahepatic cholestasis is the most common disease among metabolic diseases resulting in LT and Wilson disease is the second most common. Post-transplant immunosuppression was similar for both groups. There was no difference in both groups regarding the onset of post-transplant complications for graft type or recipient age. Biliary and portal vein complications were most particularly defined in the group with non-metabolic diseases. There was no significant difference in survival between the two groups.

Conclusion: LT is an important treatment option for acute hepatic failure and end-stage liver diseases. In addition, LT is an alternative treatment option for some metabolic diseases.

Keywords: Complications, non-metabolic diseases, metabolic diseases, liver, survival, transplantation

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Introduction

In children, liver transplantation (LT) can be performed at many indications, including malignancy and acute hepatic failure. LT is an important treatment option, especially in children with biliary atresia, progressive familial intrahepatic cholestasis (PFIC), Wilson disease and some metabolic diseases (1,2). In metabolic diseases, multiorgan failure may occur due to an accumulation of toxic metabolites in organs. In inherited metabolic diseases, LT has two main purposes: to keep the patient alive in progression to hepatic failure and to completely eliminate the underlying metabolic defect for some metabolic diseases. Some metabolic disorders cause progressive liver damage and may require LT (3). Other metabolic disorders do not cause structural liver damage, but toxic metabolites have extra hepatic effects in some diseases such as urea cycle defects, primary hypercalcemia Type I, and Crigler-Najjar syndrome Type I. (3-6). LT can be performed to relieve the enzyme deficiency if alternative treatment options are not sufficient or metabolic decompensation could not be prevented in metabolic diseases (3,7). During the last few decades, LT has become an alternative treatment in metabolic diseases. In most pediatric transplant centers, metabolic liver disease is the second most common indication for LT after biliary atresia (8,9).

Due to progress in the field, long-term survival rates of pediatric LT are now over 80% with the majority of mortalities occurring within 6 months of the transplant procedure (10,11). The survival rates for 1 and 5 years after LT in children are 77-86%, 73% and 87%, respectively (12-14). These rates may be better for children with LT for metabolic diseases. The 1- and 5-year survival rates of children with LT due to metabolic disease vary between 87-94%, 79% and 92%, respectively (15-17). The purpose of this study is to compare the treatment and follow-up results of patients who underwent LT for metabolic disease and other causes in our transplantation program.

Materials and Methods

One hundred eighty-nine patients who underwent LT between 1997 and 2015 due to metabolic diseases and acute or chronic liver failure were included in the study. This retrospective study reviewed the records of these patients. After the LT, all the recipients were followed monthly during the first six months, every three months in the next six months and after the first year they were followed up every six months.

Gender, age, age at transplantation, type of transplantation, type of donor, rejection and complications were evaluated. This study was performed retrospectively. The study was prepared in accordance with the Helsinki Declaration. An informed consent form was obtained from the patients' relatives.

Statistical Analysis

The results are expressed as mean values where indicated; a paired Student t-test was used to assess differences between the two groups. The Kaplan-Meier method was used to assess patient survival rates. Differences in survival were compared using a log-rank analysis. $P<0.05$ was considered significant. All the statistical analyses were performed using MedCalcx Software (Ostend, Belgium, https: medcalc.org; 2013) version 12.7.7.

Results

A total of 189 patients, 54% (n=102) male and 46% (n=87) female were included in the study. Fifty-six patients in the Metabolic disease group were enrolled (PFIC: 22 patients, Wilson disease: 11 patients, tyrosinemia Type I: 9 patients, familial hyperlipidemia: 4 patients, alpha 1 antitrypsin deficiency: 3 patients, glycogen storage disease (GSD) Type I: 3 patients, Crigler Najjar syndrome Type I: 2 patients, GSD Type III: 1 patient, GSD Type IV: 1 patient) in the study. Details are given in Figure 1. In the non-metabolic liver disease group; there were 133 patients (biliary atresia: 55 patients, autoimmune hepatitis: 13 patients, fulminant hepatitis: 32 patients, tumor: 10 patients, other cholestatic disease: 19 patients and others: 4 patients). The diagnosis of these patients is detailed in Figure 2.

Biliary atresia is the most common transplant indication for LT. The other important indications for LT are fulminant hepatic failure, autoimmune hepatitis and cholestatic liver diseases.

From a total of 189 patients; 29.6% (n=56) of cases were diagnosed as metabolic diseases including Wilson disease, Tyrosinemia and PFIC. Median age at diagnosis was 11.8 (1-31) months in the non-metabolic group and 2.2 (5 months-17 years) years in the metabolic group. In the metabolic diseases group, mean age at transplantation was 5.52±4.75 (6 months-17 years) years and the non-metabolic group mean age at transplantation was 5.6±5.4 (6 months-14 years) years. The characteristics of the patients diagnosed with non-metabolic and metabolic diseases are shown in Table I. The characteristics of the patients diagnosed with Metabolic diseases are shown in Table II.

Only one patient with familial hyperlipidemia and one patient with PFIC underwent a second transplant. In the group of patients with metabolic diseases (n=56), 5 cases were diagnosed with tyrosinemia Type I and developed hepatocellular carcinoma. PFIC was the most common disease among the patients with metabolic diseases who underwent LT, and Wilson disease was the second most common. In the non-metabolic group, 69.8% (n=92) of patients had a transplant from a living donor, while the rest of the patients (n=41) received a transplant from cadavers. More than two-thirds of patients with metabolic disease had a LT from a live donor. Rejection was found in 15% of those

patients with metabolic disease while the percentage of organ rejection in the other group was 18%. Post-transplant immunosuppression was similar for both groups. There was no difference in both groups regarding the onset of the post-transplant complications, graft type or recipient age. Biliary and portal vein complications were most particularly defined in the group with non-metabolic diseases. There were three patients with gastrointestinal system complications in the metabolic diseases group, however no patient developed gastrointestinal complications in the non-metabolic disease

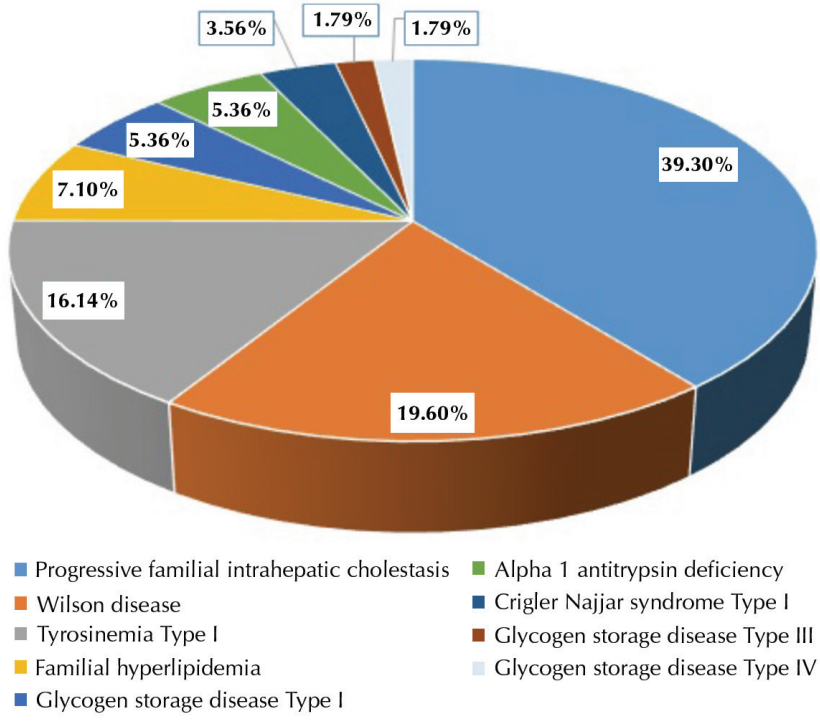

Figure 1. Metabolic diseases groups

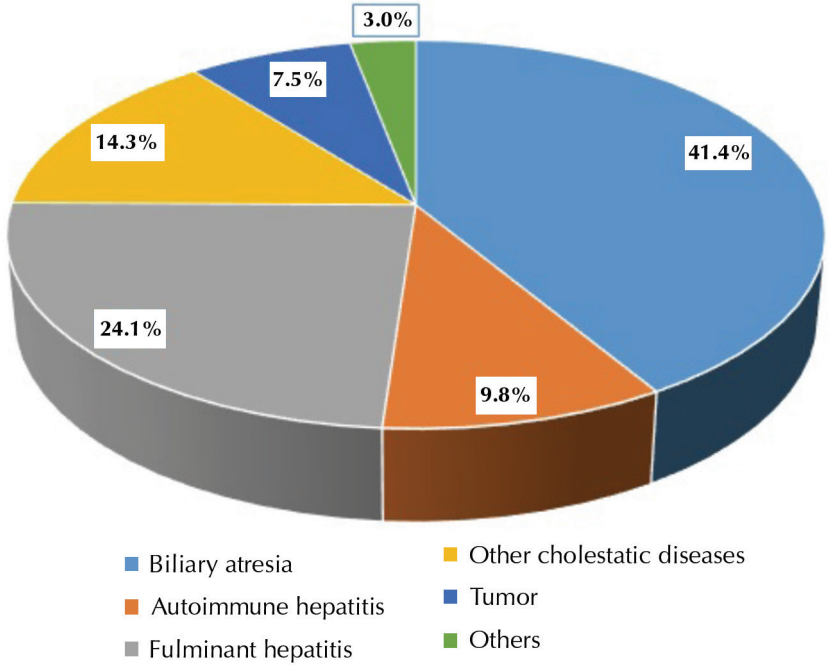

Figure 2. Non-metabolic diseases groups

group. Post-transplant complications of the patients diagnosed with metabolic diseases are given in Table III.

The rate of immunosuppressive drugs which were used for LT due to metabolic disease varied as follows: 60.7% tacrolimus, 28.5% sirolimus and 10.8% cyclosporine. The rate of immunosuppressive drugs which were used for LT due to non-metabolic disease varied as follows; 71.4% tacrolimus, 18% cyclosporine and 9.8% sirolimus. In terms of ongoing medication, no statistically significant difference was detected between the two groups.

In the non-metabolic disease group, the survival rate for the first year after transplantation was 82%, but in the fifth year this rate dropped to 79%; In the group of patients with metabolic disease, the survival rate at one year after transplantation was 80%, while in the fifth year it was 77% (Figure 3). No significant difference was detected between the two groups.

Table I. Characteristics of the patients diagnosed with non-metabolic and metabolic disorders

Group	Non-metabolic disease	Metabolic disease	p value
Gender (female/male)	64/69	30/26	0.96
Mean age at transplantation	5.6±5.4	5.52±4.75	0.92
Donor type			
- Cadaveric	40	19	0.90
- Living donor	93	37	-
- Rejection	31	5	0.44
Treatment			**0.38**
- Siklosporin	24	16	-
- Tacrolimus	95	36	-
- Sirolimus	14	4	-
Number of patients with post-transplant complications (%)	38 (28.6)	15 (26.7)	0.56

Discussion

This study reviewed the experience and long-term follow up of pediatric patients with metabolic and non-metabolic diseases who underwent LT at our center during the last 18 years. In the literature, post transplantation survival rates of patients who had inborn defects of metabolism appear to be higher, when compared to survival following transplantation for other indications, such as extrahepatic biliary atresia, acute liver failure, or post necrotic liver cirrhosis (16). Most studies regarding LT for metabolic liver diseases involve pediatric patients. In our study, the survival rate for the first year after transplantation was 82%, but in the fifth year this rate dropped to 79%; in the group of patients with metabolic disease, the survival rate one year after transplantation was 80%, while in the fifth year, it was 77%. According to our analysis, the patient survival rate was similar for children with metabolic and non-metabolic diseases. The survival rate was the same in both groups, which might be related to accompanying pre-transplant and post-transplant factors and the diseases which caused parenchymal liver disease in both groups. Pre-transplant health status affects post-transplant survival (2). The one- and five-year patient survival rates were reported as between 92% and 94% in the United States (18) whereas

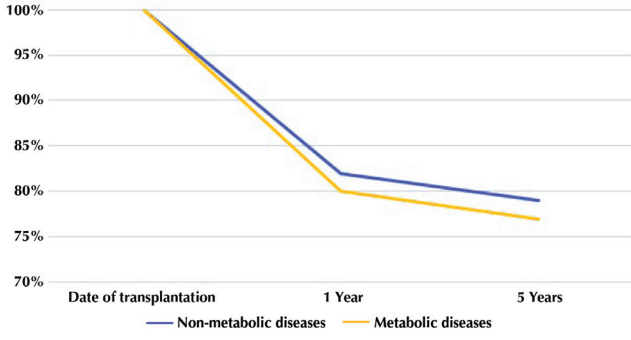

Figure 3. Survival rate of metabolic and non-metabolic diseases

Table II. Characteristics of the patients diagnosed with metabolic disorders

	CNS Type I	GSD Type III	Wilson disease	PFIC	Alpha 1 antitrypsin deficiency	GSD Type I	GSD Type IV	Tyrosinemia	Hyperlipidemia
Number of patients	2	1	11	22	3	3	1	9	4
Number of living patients	2	1	8	15	3	3	1	8	4
Median age (year)	2	4	12.44±4.04	3.9±2.64	0.8±0.28	8.3±4.93	4	2.75±2.41	5.5±3.5
Donor type numbers, cadaveric/ living	1/1	-/1	5/6	8/14	-/3	1/2	-/1	1/8	-/4

CNS: Crigler-Najjar syndrome Type I, GSD: Glycogen storage disease, PFIC: Progressive familial intrahepatic cholestasis

Sze et al. (19) reported survival rates of 91% and 86%, respectively, in the United Kingdom. The survival rates at 1, 5, and 10 years in these studies were similar to other studies. (16,17,20).

Arnon et al. (21) showed that survival rates of their patients with metabolic and non-metabolic diseases were 94.6% and 90.7% at one year respectively and 88.9% and 86.1% respectively at year five. The cumulative survival rates in pediatric patients with non-metabolic disease were 91.9%, 87.2%, and 85.8% at one, five, and 10 years, respectively (22). The survival rate was lower in both the metabolic and non-metabolic disease groups in our study, which was different from the study by Kayler et al. (15). Kayler et al. (15) and Arnon et al. (21) had patients without metabolic disease due to parenchymal liver disease, which may account for the lower survival rate in our study. However, we had more patients with PFIC and Wilson Disease than other studies. The study by Kayler et al. (15) conspicuously contained a lot of patients with alpha 1 antitrypsin deficiency (n=261). However, Kayler et al. (17) defined the metabolic group as only patients with biliary atresia, which meant that he compared biliary atresia to a non-metabolic group. These results may be due to the involvement of patients with LT due to tumor, autoimmune hepatitis, fulminant hepatitis, which might worsen the outcomes compared with postoperative transplantation due to BA. (23,24). We classified those patients with PFIC into the metabolic disease group, which may be why the survival rate is lower in our study than in other studies.

In our work, in accordance with other studies, we found that Pediatric End-stage Liver Disease scores were statistically significantly lower in children with metabolic diseases (25). As a result of very good survival rates, complications after LT can be seen in children. In the Arnon et al. (21) study, gastrointestinal and hematological complications were more frequent. Peeters et al. (1) reported less gastrointestinal complications post-LT in patients with metabolic disease than in those with BA. In our study, autoimmune hemolytic anemia was more common in patients with metabolic disease after transplantation than in the non-metabolic disease group. Gastrointestinal complications were more common in those patients in the non-metabolic liver diseases group (1). According to Kasahara et al. (24), seizure was one of the common problems, related with patients with metabolic diseases. The effects on systems were different from each other in metabolic diseases. In our study, we did not observe any seizure after LT.

In terms of acute rejection rates, we found a rate of approximately 15%, whereas Rosencrantz et al. (25) found a rate of approximately 22%, which was more than ours. This fact could be related to different treatment procedures. In our study, 61% of patients were on tacrolimus treatment.

The present study has several limitations that are the result of its single-center, retrospective design. In our study, biliary complications were the most common cause of post-transplant complications in both groups, the latter was portal vein complications. The reason which may cause a difference from the results of the other studies might be due to the low cadaver rates, especially among child cadavers, and the tendency to prefer living donors for LT in our country.

Conclusion

In hereditary metabolic diseases, orthotopic LT has very good survival outcomes. These children should be carefully monitored for the timing of transplantation. LT is one of the curative treatments in hepatic failure and end stage of liver failure. Also, we want to mention that LT is an alternative treatment for some metabolic diseases.

Table III. Post-transplant complications of the patients diagnosed with metabolic diseases

	CNS Type I	GSD Type III	Wilson disease	PFIC	Alpha 1 antitrypsin deficiency	GSD Type I	GSD Type IV	Tyrosinemia	Familial hyperlipidemia
Number of patients	2	1	11	22	3	3	1	9	4
Biliary complications	-	1	5	2	-	-	-	-	-
Renal stone	-	-	-	-	-	1	-	-	-
Portal thrombosis	-	-	-	1	-	-	-	-	-
HLH	-	-	-	1	-	-	-	-	-
CRF	-	-	-	1	-	1	-	-	-
OIH	-	-	-	1	-	-	1	-	-

CNS: Crigler-Najjar syndrome Type-I, GSD: Glycogen storage disease, PFIC: Progressive familial intrahepatic cholestasis, HLH: Hemophagocytic lympho histiocytosis, CRF: Chronic renal failure, OIH: Autoimmune hemolytic anemia

Ethics

Ethics Committee Approval: This study was performed retrospectively. The study was prepared in accordance with the Helsinki Declaration.

Informed Consent: An informed consent form was obtained from the patients' relatives.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: M.K., O.E., F.Ç., S.A., M.K., Concept: S.A., Design: S.A., M.K., E.C., S.K., Data Collection or Processing: M.K., E.C., Analysis or Interpretation: M.K., Literature Search: M.K., E.C., S.K., Writing: M.K., E.C.

Conflict of Interest: There is no conflict of interest.

Financial Disclosure: The authors declare they received no financial incentive in writing this research.

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Original Article

Evaluation of Cardiovascular Involvement and Cytokine Levels in Patients with Mucopolysaccharidosis

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ABSTRACT

Aim: Cardiovascular involvement is common in patients with mucopolysaccharidoses (MPS). In this study, we investigated the effects of the markers involved in vascular endothelial injury pathogenesis [transforming growth factor β- (TGF-β)], interleukin-6 (IL-6), IL-10, high sensitive-C reactive protein (hs-CRP), vascular endothelial growth factor (VEGF), N-terminal pro-Natriuretic peptide (NT-proBNP) and the clinical, laboratory and echocardiographic findings of the patients.

Materials and Methods: A total of 37 patients (5 MPS I, 4 MPS II, 2 MPS IIIa, 4 MPS IIIb, 14 MPS IVa, 8 MPS VI) and 32 controls with similar age and sex were included in the study.

Results: Corneal clouding was seen in 29 (78%) patients. There were 23 (62%) patients with organomegaly, and 28 (75%) patients with hearing loss. When the groups were compared in terms of NT-proBNP, hs-CRP, TGF-β, IL-6, IL-10 and VEGF levels, there was a statistically significant increase in the patient group for NT-proBNP and VEGF (p=0.04, p=0.03, respectively). The carotid intima media thickness was statistically significantly higher in the patient group (p<0.001). The left ventricular diastolic diameter was significantly higher in the patient group (p=0.009), intraventricular septum thickness was significantly higher in the patient group (p<0.001). The E/A ratio was significantly lower in the patient group (p<0.001).

Conclusion: Cardiac involvement in MPS patients is a major cause of mortality and morbidity. It is thought that cytokines, proinflammatory markers are elevated in patients with vascular damage like other lysosomal diseases. There is a need for further studies to determine biomarkers for vascular involvement.

Keywords: Mucopolysaccharidoses, carotis intima media thickness, vascular involvement, cytokines

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Introduction

Mucopolysaccharidoses (MPS) are lysosomal storage disorders that occur when there is a deficiency of the enzymes responsible for the catabolism of glycosaminoglycans (GAG). Clinical symptoms develop as a result of the deposition of non-degraded GAGs in certain organs, as well as in all tissues. In total, seven types of MPS (I, II, III, IVA, VI, VII and IX) have been defined, relating to 11 different enzyme deficiencies. Its incidence is known to be 2-5 per 100.000 live births (1-3).

Cardiac pathologies may develop as a result of GAG deposition in the myocardium, cardiac valves and the myointima of the coronary arteries (4,5). Severe valvular stenosis or failure may also occur due to the thickening and calcification of the mitral and aortic valves (4,5). Cardiac involvement is more severe and occurs in the earlier stages of the disease in patients with Type I MPS and is a significant cause of mortality and morbidity in MPS patients. Histopathological investigations have identified GAG depositions in the cardiac valves, endocardium and myocardial coronary artery, and also in the aorta and conductive systems of MPS patients (6,7). Heparan-, dermatan-, chondroitin- and keratan-sulfate based GAGs are found naturally in the structure of cardiac valves and in the giant vessels, although among these, dermatan-sulfate is the most common, meaning that cardiac valve involvement is common in patients with Type I, II and VI MPS (8). Cardiovascular involvement is common among MPS patients. While it may occur with all types of MPS, it more commonly accompanies and occurs in the earlier stages of the disease in MPS I, II and VI. That said, there is only limited data on the frequency of cardiac involvement in MPS VII, as MPS VII itself is a very rare condition (9,10). Cardiac involvement may be seen in the form of cardiac valve thickening, left ventricular hypertrophy, coronary artery disease, rhythm disorders or other vascular involvements (11).

There is an increasing need to develop novel non-invasive means of predicting potential cardiovascular side effects. Several studies have identified a correlation between peripheral vascular endothelial dysfunction and coronary endothelial dysfunction, and the measurement of carotid artery thickness (C-IMT) is a useful method for the evaluation of peripheral vascular endothelium. In a study investigating the relationship between GAG metabolism disturbances and atherosclerosis, a complex process led by proteoglycans and involving a GAG metabolism was reported to have resulted in atherosclerosis and endothelial dysfunction (12). In the literature, histopathological similarities have been reported between the coronary artery lesions detected in an MPS I patient and the atherosclerotic changes observed in adults (13). An autopsy sampling of that patient showed that negative-loaded GAGs caused the activation of TGF-β in the extracellular matrix of the myocardium, which may have led eventually to hypertrophic cardiomyopathy (14).

The present study makes a comprehensive evaluation of cardiac function and vascular endothelial involvement in patients followed-up with a diagnosis of MPS. To this end, we measured the levels of the markers; transforming growth factor β (TGF β), interleukin-6 (IL-6), IL-10, high sensitive C-reactive protein (hs-CRP), vascular endothelial growth factor (VEGF), N-terminal-pro-Natriuretic peptide (NT-proBNP), involved in the pathogenesis of vascular endothelial damage, and investigated potential correlations between these levels and the clinical and laboratory findings of MPS patients, as well as obtaining information about peripheral vascular involvement based on C-IMT thickness and biochemical markers. The information collected in this study may provide clinicians with more treatment options for MPS patients, and the markers may also have potential for use during the follow-up of MPS patients.

Materials and Method

The study included MPS patients followed-up by the pediatric metabolism and nutrition outpatient clinics, and who also provided consent for participation in the study. The clinical and laboratory findings of the patients were retrieved from their medical records, while ECG and echocardiographs, as well as C-IMT measurements, were obtained as a routine part of patient follow-up in outpatient clinics. Data was collected retrospectively from medical records. Age- and gender-matched healthy individuals without any known chronic disorders were included in the study as a control group after providing informed consent for participation. C-IMT measurements of the controls were obtained. All blood samples were centrifuged and stored at -20 °C. TGF-β, IL-6, IL-10, hs-CRP, VEGF, and NT-proBNP levels were measured in these blood samples using ELISA kits.

A complete echocardiography evaluation by a padiatric cardiologist who was blinded to the diagnoses was performed in all patients with a two-dimensional M-Mode and Doppler Echocardiogram using a Vivid 9 system (GE Vinmed, Horten, Norway) M5Sc and 11L transducer. All patients were kept in a left decubitis position during examination and the measurements were made using techniques according to the recommendations of the American Society of Echocardiography (15). High-resolution B-mode ultrasonography was performed in all patients on the right carotid artery and C-IMT was calculated by taking the mean of three measurements. The details of the measurements were given in a previous study (16,17).

The study was approved (approval number: 14-3.1/1 4.4.2014) by the Ethics Committee of Ege University Faculty of Medicine and was supported by Ege University Scientific Research Projects Coordination, İzmir, Turkey.

Statistical Analysis

SPSS 22.0 for Windows was used for the statistical analysis. Qualitative data are presented as counts and percentages. Quantitative data were given as mean ± standard deviation for normally distributed data or otherwise median and range. Student's t-test was used to compare the differences between the patient and control groups for normally distributed data. Mann-Whitney U test was used for non-parametric parameters. For all comparisons, p values lower than 0.05 were considered statistically significant.

Results

A total of 37 patients diagnosed with MPS were included in the study. The mean age of the patient group was 11.3±6.2 years (2.5-35 years), while the mean age of the healthy control group was 12.3±6.0 (3-36 years) years which consisted of 32 participants. The MPS patient group comprised 18 (48%) females and 19 (52%) males, while the healthy control group comprised 16 (50%) female and 16 (50%) male participants. The characteristics of the patients with MPS are detailed in Table I. The age and gender distribution within the two groups were not significantly different.

The study included a total of 37 patients diagnosed with MPS (5 MPS I, 4 MPS II, 2 MPS IIIa, 4 MPS IIIb, 14 MPS IVa, 8 MPS VI) and 32 age- and gender-matched healthy controls. The majority of patients included in the study had been diagnosed with MPS IVa. Disease-related enzyme levels decreased, and urinary GAG excretion increased in all patients, and all patients had dysostosis multiplex findings. Corneal clouding was noted in 29 patients, and two patients received corneal transplants. In total, 23 (62%) patients had organomegaly and 28 (75%) patients had hearing loss. The systemic involvement of the patients with MPS is given in Figure 1. The most common location of cardiac involvement was the mitral valve in cases of single valve involvement. Simultaneous involvement of the mitral and aortic valves was present in 15 (47%) patients and was most often seen as the co-involvement of the mitral and aortic valves (Figure 2). Echocardiographic investigations revealed normal findings in four patients, three of which had been diagnosed with MPS IA. Furthermore, four patients had increased left ventricular wall thickness and left ventricular dysfunction; and one patient experienced a marked pulmonary gradient increase and an enlargement of the right cardiac structure.

In the patient group, the median NT-proBNP level was 44.4 (range: 17.6-495) pg/mL, the median VEGF level was 518.5 (range: 95-6359) ng/L. In the healthy control group, the median NT-proBNP level was 29.2 (range: 19.6-81.53) pg/mL, the median VEGF level was 235.0 (range: 90.0-1796) ng/L. When the NT-proBNP, hs-CRP, TGF-β, IL-6, IL-10 and VEGF levels were compared between the two groups, significant elevations were noted for NT-proBNP and VEGF in the patient group when compared to the healthy controls (p=0.04, p=0.03, respectively) (Table II). Cytokine levels of the patients and control group are given in Table I.

The patients were further classified into two groups, group 2 [(MPS I, II, IV and VI (n=29)], which underwent enzyme therapy and group 1, which underwent no enzyme therapy [(MPS IVA-2 patient-pre-treatment and diagnosed with MPS III (n=6)]. NT-proBNP, hs-CRP, TGF-β, IL-6, and IL-10 levels were not found to be significantly different between the patients who were receiving enzyme therapy and those who were not (Table III).

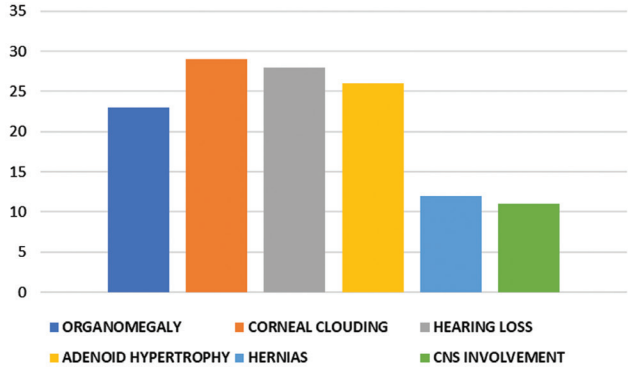

Figure 1. Clinical findings of the patients

Table I. Characteristics of the patients with mucopolysaccharidosis

MPS type	Number	Gender, (M/F)	Age (years), (min-max)	Age at diagnosis (years), (min-max)	Alive / treatment with ERT
I	5	2/3	8.7±5.8 (2.5-16.5)	4.2±6.0 (0.9-15)	4/4
II	4	4/-	8.6±4.2 (4-13.5)	3.1±1.7 (1.0-5.0)	3/3
IIIa	2	2/-	9 and 12.5	2 and 4	-
IIIb	4	2/2	13.7±1.8 (12-16)	6.0±2.0 (3-7)	1/-
IV	14	6/8	12.1±8.4 (4.3-35)	7.6±8.5 (0.5-32)	12/11
VI	8	4/4	11.8±4.4 (6.5-17)	5.1 (2.2) 2.5-9.0	7/7

M: Male, F: Female, min: Minimum, max: Maximum, ERT: Enzyme replacement therapy, MPS: Mucopolysaccharidosis

The carotid intima media thickness was significantly higher in the patient group when compared to the controls (p<0.001) (Figure 3), and the left ventricular diastolic diameter was also significantly higher in the patient group (p=0.09). Intraventricular septum thickness was significantly higher in the patient group (p<0.001). The E/A ratio was significantly lower in the patient group (p<0.001). The right ventricular end-diastolic diameter (RVd) was also higher in the patient group than in the controls, although not to a statistically significant degree (p=0.05). The right ventricular end-systolic diameter was significantly higher in the patient group (p<0.001). The echocardiographic evaluations results of the patients are given in Table IV.

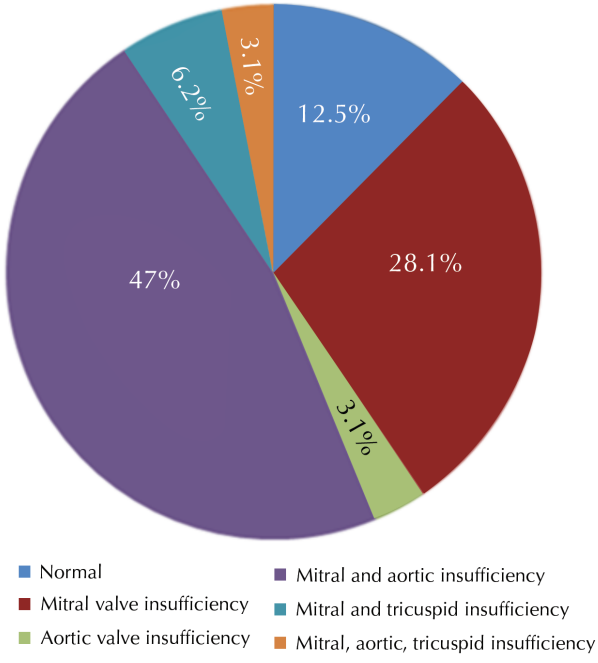

Figure 2. Valvular involvement of the patients

Discussion

In our study, we evaluated proinflammatory cytokine levels in different types of MPS and compared with healthy controls. Progressive valvular involvement is the most common cardiac pathology among MPS patients (60-90%) (14), and dysfunction due to the thickening of the cardiac valves has been reported previously in 80% of MPS I patients by Pastores et al. (18), and in 57% of MPS II patients (19). Several studies have shown that the mitral valve was the most commonly involved valve, and valve deficiency was more common than obstruction (4). In their study of MPS Type VI patients, Azevedo et al. (20) reported that the mitral valve was the most common location of cardiac involvement, with mitral failure noted in more than 95% of their patients. The mitral valve was also the most commonly

Table II. Cytokine levels of the patient and control group

	Patients median (range)	Control median (range)	p value
NT-proBNP (pg/mL)	44.4 (17.6-495.0)	29.2 (19.6-81.53)	0.04
Hs-CRP (mg/L)	7.0 (2.6-75)	4.8 (2.5-26.6)	0.37
TGF-β (ng/mL)	160.6 (76-2079.0)	109.0 (78.6-480.3)	0.65
IL-6 (ng/L)	85.8 (41.0-995.0)	72.5 (45.7-237)	0.66
VEGF (ng/L)	518.5 (95-6359)	235.0 (90.0-1796.0)	0.03
IL-10 (pg/mL)	340.5 (91-3435)	203.5 (111.0-881.0)	0.45

NT-proBNP: N-terminal pro-natriuretic peptide, hs-CRP: High sensitive C-reactive protein, TGF-β: Transforming growth factor-β, IL: Interleukin, VEGF: Vascular endothelial growth factor

Table III. Cytokine levels of the treated and untreated patients

	Untreated patients median (range)	Treated patients median (range)	p value
Number of Patients	8	29	-
NT-proBNP (pg/mL)	42.8 (91-3435)	44.4 (17-495)	0.8
Hs-CRP (mg/L)	5.3 (2.9-53)	7.3 (2.6-75)	1
TGF-β (ng/mL)	155 (86.8-866.0)	162.1 (76.8-2079)	0.8
IL-6 (ng/L)	77.6 (54.2-494.7)	89.1 (41.0-995.0)	1
VEGF (ng/L)	633.5 (116-4699)	467.0 (95-6359)	0.7
IL-10 (pg/mL)	253.5 (151-1461)	354.5 (91-3435)	0.9

NT-proBNP: N-terminal pro-natriuretic peptide, hs-CRP: High sensitive C reactive protein, TGF-β: Transforming growth factor-β, IL: Interleukin, VEGF: Vascular endothelial growth factor

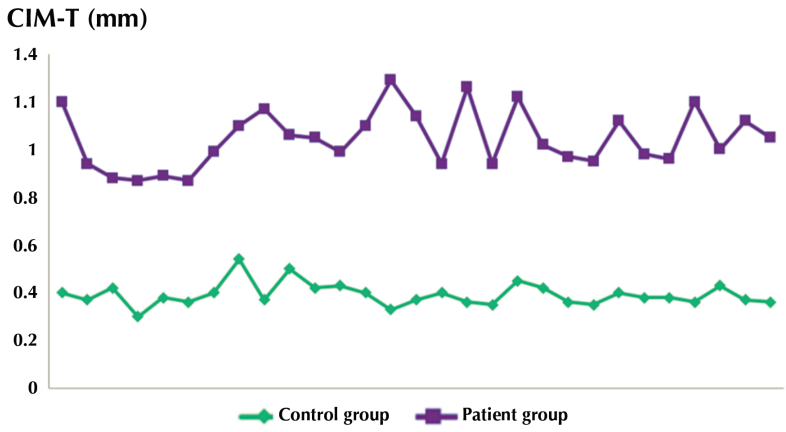

Figure 3. C-IMT levels of the patients and control groups
C-IMT: Carotid artery thickness

Table IV. Cardiac evaluation of the patients with echocardiography

	Patients mean (min-max)	Control mean (min-max)	p value
Heart rate (minimum)	109.6±19.4 (75-163)	81±9.7 (65-105)	<0.001
LVDd	32.7±5.58 (23.1-44.0)	29.6±2.91 (23.7-37.8)	0.009
LVDs	19.1±5.4 (12.1-31.8)	18.8±2.09 (14.5-22.6)	0.7
RVDd	18.5±4.1 (6.5-24.5)	16.9±1.3 (15-20)	0.05
RVDs	13.6±2.6 (9.5-19.6)	11.1±1.8 (7.8-15.6)	<0.001
IVSDd	6.7±2.3 (4.2-16.5)	3.7±0.5 (2.9-5)	<0.001
E/A ratio	1.1±0.3 0.6-2.1)	1.5±0.1 (1.2-2)	<0.001

Min: Minimum, Max: Maximum, LVDd: Left ventricular diameter in diastole, LVDs: Left ventricular diameter in systole, RVDd: Right ventricular diameter in diastole, RVDs: Right ventricular diameter in systole, IVSDd: Intraventricular diameter in diastole

involved valve in the present study. While 15 patients had mitral and aortic valve involvement, four had increased left ventricular size and dysfunction, and one patient had a marked pulmonary gradient increase and enlarged right cardiac structures.

C-IMT is a useful method for the evaluation of peripheral vascular endothelium. In another study, Wang et al. (21) compared the C-IMT of MPS patients and healthy controls, evaluating 16 MPS patients based on neck C-IMT measurements and echocardiographic evaluations. The widths of the left main coronary left anterior descending and right coronary artery were not significantly different when compared to the control group, although mitral valve and aortic failure, as well as left ventricular dilatation, were found to be significantly more common in the MPS patients than in the control group. C-IMT levels were also elevated in MPS patients compared to the controls. The authors underlined the need for future studies investigating the correlation between C-IMT and arterial elasticity, and potential biomarkers for vascular dysfunction (12). In the present study, the C-IMT levels in the patient group were significantly higher than in the healthy controls.

In the literature, histopathological similarities have been reported between the coronary artery lesions detected in a MPS I patient and the atherosclerotic changes observed in adults. An autopsy sampling of that patient showed that negative-loaded GAGs caused the activation of TGF-β in the extracellular matrix of the myocardium, which may have led eventually to hypertrophic cardiomyopathy (13). In a study carried out by Yano et al. (22) on an autopsy specimen of a deceased MPS I patient, "phosphorylated Smad 2" immunofluorescent staining revealed a significant signal increase in the vascular wall and the myocardium when compared to the control samples. This supports the increased TGF-β signal (22). In another study investigating the relationship between TGF-β and arterial pathology in MPS I animal models, immunohistochemically investigations of the sclerotic vessels showed TGF-β positivity and an increase in fibronectin (23). According the literature findings, we expected to find a difference between the groups for TGF-β levels, but we could not find any significant difference for TGF-β levels between the groups. However, the levels were higher in the patient group. With the investigation

of a larger number of patients, we think that a significant difference may be detected.

VEGF is a family of multifunctional growth factors that may have specific effects particularly on the endothelial cells. The family has six sub-members that play roles in the proliferation, migration and differentiation of endothelial cells. The expression of VEGF increases following hypoxia associated with an obstruction of the cardiac vessels (24). The relationship between VEGF and obstructions of the coronary or peripheral arteries has to date not been investigated specifically in MPS patients. An earlier study investigated the role of VEGF in fabry disease, which is another lysosomal storage disorder, and VEGF levels were found to be elevated in patients with fabry disease. Based on their results, the authors of the study underlined that VEGF may be developed as a response to vascular damage in lysosomal diseases (25). There have been no studies to date investigating VEGF levels in MPS patients. In the present study, we found that VEGF levels were significantly elevated in patients diagnosed with MPS.

Proinflammatory cytokines IL-6 and hs-CRP play a role in atherosclerotic plaque destabilization (26). A study involving 279 patients with atherosclerosis showed that hs-CRP, IL-6 and IL-10 were associated with C-IMT levels in atherosclerosis, and C-IMT and NT-proBNP were also associated with sudden cardiac events (27). In a study performed by Krecki et al. (28), hs-CRP and NT-proBNP levels were found to rise in proportion to the severity of the disease in patients with coronary artery atherosclerosis. Donida et al. (29) investigated oxidative stress and inflammation markers in 17 patients with MPS Type IVA who were undergoing enzyme replacement therapy and found that IL-6 levels were higher in the MPS IVA patients than in the healthy controls. In our study, we detected a statistical difference between groups for NT-proBNP levels. The levels of hs-CRP, IL-6 and IL-10 were all higher in MPS patients than in the control group but there was no difference between groups.

The levels of the biomarkers investigated in this study were found to be elevated in MPS patients. Only NT-proBNP and VEGF levels were statistically different. C-IMT was also significantly elevated. These findings could be due to the inflammatory processes atherosclerosis developed in MPS patients. To the best of our knowledge, cytokines and proinflammatory markers have not been evaluated together in different types of MPS patients.

Conclusion

Cardiovascular involvement is a significant cause of mortality and morbidity in MPS patients. As is the case with other lysosomal diseases, MPS patients can be expected to have increased levels of cytokines and proinflammatory markers associated with vascular damage. The levels of biomarkers investigated in the present study were found to be elevated in MPS patients. Additional studies are required to investigate the potential use of markers of vascular involvement during the follow-up of MPS patients. In line with the earlier studies with similar findings, new treatment options need to be considered that suppress the inflammatory process induced by deposition.

Ethics

Ethics Committee Approval: The study was approved by the ethics comity of Ege University Faculty of Medicine (approval number:14-3.1/1 4.4.2014).

Informed Consent: Consent form was filled out by all participants.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: E.C., R.E.L., E.S., M.D., Concept: E.C., M.Ç., S.K.U., Design: E.C., M.Ç., Data Collection or Processing: E.C., M.K., M.D., Analysis or Interpretation: E.C., M.Ç., S.K.U., E.S., Literature Search: E.C., M.K., M.KA., Writing: E.C.

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Original Article

Sleep Characteristics of Pediatric Burn Patients

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ABSTRACT

Aim: Soft tissue injuries which happen because of high heat, chemicals etc. are called burns. Sleep is a complex behavior regulated by the interaction of anatomical and neurochemical areas in the central nervous system. After being burnt; patients are exposed to many stressors that cause sleep interruption and decrease sleep quality. The purpose of this study was to determine sleep characteristics, sleeplessness and sleep habits in pediatric burn patients.

Materials and Methods: This descriptive study was carried out on 96 children who were 2-6 years old in the pediatric surgery department/burn unit for a period of between 2-7 days. For data collection, a demographic data collection form and a sleep problems characteristics and identification survey, which was created by the researchers, were used. Data were collected via face to face interviews with mothers. Questions were asked in order to compare pre- and post- burn sleep quality.

Results: Of the children, 53.1% were male and their mean age was 3.36±1.39 years. There was a significant difference between pre- and post-burn periods on children's sleeping and waking hours, total and night sleep duration, total daily sleep duration, existence of sleep interruption and number of interruptions, trouble of falling asleep and sleep latency, being tired in the morning, forcing the children to wake up and also the duration of waking up.

Conclusion: With the knowledge about the importance of sleep, attention should be paid to the sleep of children that we are providing care to. Supportive environmental regulations should be made to improve the quality of sleep in hospitals.

Keywords: Pediatrics, burn, sleep

Introduction

Pediatric burns are the third most common cause of accident-induced mortality following motor vehicle accidents and drowning (1). In the United States of America, approximately 300 children are admitted to the hospital for burn treatment and two of them die each day (2). Burn injuries lead to sudden changes in the metabolism including hypermetabolism and catabolism (3). The reasons for the increase in the metabolic rate are the effects of inflammation on the thermoregulation system after being burnt and heat loss caused by evaporation (4). If 5% or more of the total body surface area is burned in adults, this leads to an increase in the metabolic rate from 118% to 210% (5).

As in all traumas, children are more vulnerable to burn injuries than adults, which is due to physiological and anatomical differences between adults and children. Neurological systems that have not completed their development in children may not tolerate the increase in the level of norepinephrine, one of the main neurotransmitters. Children's total body surface area is more than that of adults. Accordingly, their metabolic rates are higher. Due to their thinner dermis and larger body surface area compared to those of adults, they are at

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a greater risk of developing complications in burn traumas (6).

After suffering a burn, patients are exposed to many stressors that lower sleep quality and interrupt sleep (7). Treatment-associated factors such as fixation methods, physiological factors, therapeutic interventions, diagnostic procedures, mechanical ventilation, sedatives, analgesic and anesthetic drugs are known to affect sleep. Burn patients are exposed to many environmental stimuli such as light, wound dressings, treatment alarm sounds of the devices, and sounds from infusion pumps, phones, pagers and televisions which disrupt their daily routine (8). Thermal injuries cause an increase in sleeplessness, and significant reductions in non-REM sleep (stage 3 and 4) and REM sleep (9). Typically, in burn patients, increases are observed in the metabolic rate, protein degradation and weight loss (10). Sleeplessness can increase metabolic rate and catabolism as well (11). Catabolic hormones like catecholamine and cortisol increase after the burn or sleeplessness (10). Post-burn or with sleeplessness, impaired glucose tolerance can occur too (12,13); however, the amount of growth hormone decreases (14).

Although there are a lot of studies about the effects of insomnia and burns on the metabolic variables, there are only limited studies to determine sleep characteristics, sleeplessness and sleep habits. This present study was planned to determine sleep characteristics, sleeplessness and sleep habits in pediatric burn patients.

Materials and Methods

Type of Study: The study was designed as a descriptive one.

Setting: The study comprised 96 children hospitalized in the pediatric burn unit of a university hospital. As the children's burn-induced pain was intense in the first days post-burn, and because they were not expected to overcome the burn shock immediately, the data were gathered at one time between the 2nd and 7th days of their stay in the hospital.

Inclusion Criteria: Patients who were hospitalized at the burn clinic (but not in burn intensive care unit) for a minimum of 2 and a maximum of 7 days, between the ages of 24 and 72 months, and who had no surgery because of the burn, were conscious and whose parents volunteered to participate in the study were included in the study.

Implementation of the Study: The data of the study was collected between April 2015 and April 2016. All the parents gave their written consent before the study was started. Data were obtained with the survey method through face-to-face interviews with mothers. In order to determine the effect of the burn on sleep, the child's sleep patterns before and after burn injuries were compared. When the data were collected, questions on sleep problems were asked in such a way as to compare the child's condition prior to the burn at home, and post-burn in the hospital.

Data Collection Tools

Sociodemographic Characteristics Questionnaire: The socio-demographic Characteristics Questionnaire was developed by the researchers to collect socio-demographic data about the children in the study. The questionnaire has 28 items questioning the characteristic of the burn and the child's age.

Sleep Characteristics and Problems Identification Survey: Questions on the children's sleeplessness were prepared by the researcher through a literature review (15-18). In this survey, questions about sleep duration, the number of sleep disturbances, the causes of insomnia, the possibility of drowsiness and sleeping habits of the child were asked. The questions in the survey were asked both to determine the child's previous situation prior to the burn and post-burn. Thus, it was hoped to determine whether or not the child had a sleeping problem prior to the burn.

Ethics: This study's procedure was approved by the Institutional Review Board of İzmir Katip Çelebi University Non-interventional Clinical Studies Institutional Review Board (approval no: 2015/112). All voluntary participants were informed that they could withdraw from the study at any time at the beginning of the study. A consent form was filled out by all participants.

Statistical Analysis

The data obtained from the study were analyzed using the SPSS version 21 (2012). Participating patients' socio-demographic characteristics were given as number and percentage distributions. To analyze the mean scores obtained from the patients and their parents according to their socio-demographic characteristics, the compliance with normal distribution analysis was performed, and the dependent t-test, Friedman test and McNemar's test were used. Statistical significance was established as a p value <0.05.

Results

The participating children's mean age was 3.36±1.39 and of the children 53.1% (n=51) were male (Table I). The part of the body exposed to burns most in the children participating in the study (10.4%, n=10) was the "hands". Of the children participating in the study, 79.2% (n=76) had second-degree burns. The mean percentage of burns was 8.12%±5.61 (minimum: 1%, maximum 30%). Of the causes of burns in children, 47.9% were (n=46) hot water (Table II).

There was a statistically significant difference between the durations of night sleep ($t=13.644$, $p<0.001$), the times

the children woke up (t=10.023, p<0.001) and the duration of sleeps per day on pre-post burn periods (t=8.715, p<0.001) (Table III).

There was a statistically significant difference between the times the children went to sleep (F: 12.938, p<0.001) and the number of the children who had interruptions of sleep (p<0.001) and the number of the sleep interruptions in the pre- and post-burn periods (F: 55.000, p<0.001). There was a statistically significant difference between falling asleep patterns (p<0.001), and the durations of falling asleep in the pre- and post-burn periods (F: 22.349, p<0.001) and mothers' thoughts about having trouble while awakening the children (p<0.001) and in the terms of the mothers' statements that their children had sleep problems (p<0.001) (Table IV).

The sleep habits of the children in the pre-post burn periods were shown, and the pre-post burn periods were

Table I. Socio-demographic characteristics of children

Socio-demographic characteristics		**n**	**%**
Gender	Girl	45	46.9
	Boy	51	53.1
Age, Mean: 3.36±1.39, Min: 2, max: 6	2 years	40	41.7
	3 years	21	21.9
	4 years	17	17.7
	5 years	3	3.1
	6 years	15	15.6

Min: Minimum, Max: Maximum

Table II. Characteristics of burn wound

Characteristics		**n**	**%**
Location of burn	Hands	10	10.4
	Genital Area	9	9.4
	Feet	8	8.3
	Legs	7	7.3
	Arms	5	5.2
	Head	5	5.2
	Torso	3	3.1
	More than one body part	49	51.1
Degree of the burn	2nd degree	76	79.2
	3rd degree	20	20.8
Percentage of the burn Mean: 8.12%±5.61% Min: 1% Max: 30%	1%-10%	74	77.1
	11%-20%	18	18.8
	21%-30%	4	4.2
Burn factor (cause of the burn)	Water	46	47.9
	Oil	2	2.1
	Electricity	1	1.0
	Stove	4	4.2
	Flame	6	6.3
	Tea	30	31.3
	Food	4	4.2
	Others	3	3.1

Min: Minimum, Max: Maximum

Table III. Pre- and post-burn sleep characteristics-I

		Mean	**SD**	**Min-max values**	**t**	**p value**
Wake up time in the morning	Pre-burn	8.48 a.m.	1.14	Min: 5 Max: 11	10.023	<0.001
	Post-burn	6.96 a.m.	1.09	Min: 1 Max: 9		
Duration of night sleep	Pre-burn	10.83 hours	1.13	Min: 6 hours Max: 12 hours	13.644	<0.001
	Post-burn	7.44 hours	1.79	Min: 3 hours Max: 12 hours		
Duration of daytime sleep	Pre-burn	1.46 hours	1.07	Min: 0 hours Max: 3 hours	-1.307	0.194
	Post-burn	1.67 hours	1.01	Min: 0 hours Max: 6 hours		
Duration of daily sleep	Pre-burn	11.56 hours	1.40	Min: 8 hours Max: 15 hours	8.715	<0.001
	Post-burn	9.13 hours	2.07	Min: 4 hours Max: 15 hours		

SD: Standard deviation, Min: Minimum, Max: Maximum

compared from this aspect. There were statistically significant differences between the children's pre- and post-burn period sleep habits in the following circumstances: having difficulty falling asleep at night (p<0.001), having difficulty falling asleep at night again after waking up (p<0.001), taking relaxants when he/she wakes up at night (p=0.004), craving for food or drink during the night (p<0.001), fear of sleeping alone (p<0.001), fear of sleeping in the dark (p<0.001), being anxious and restless during the day because of not having enough sleep during the previous night (p<0.001), willing to be read books or listen to a lullaby before going to sleep (p<0.001), feeling restless while sleeping (p<0.001), waking up screaming, crying, and waking up due to a nightmare (p<0.001) (Table V).

Discussion

In this study, it was found that children's mean duration of night sleep altered in the post-burn period and they went to sleep later than usual in the hospital. Similarly; Bisogni et al. (19) determined that while the number of the children who slept less than 5 hours at home increased by 4.7% after hospitalization, the number of the children who slept 9-11 hours at home decreased by 5.9% after hospitalization (19). In the same study, the mothers put their children to sleep later in the hospital. While

Table IV. Pre-post burn sleep characteristics-II

		Pre-burn		Post-burn			p value
		n	%	n	%		
What time does the child to go to sleep?	7.00 p.m.-9.59 p.m.	8	8.3	12	12.5	12.938**	<0.001
	10.00 p.m.-11.59 p.m.	78	81.3	43	44.8		
	After 12 midnight	10	10.4	41	42.7		
Does the child have interruptions of sleep?	Yes	61	63.5	92	95.8	*	<0.001
	No	35	36.5	4	4.2		
How many times does the child have interruptions of sleep?	Once	32	32.5	5	5.2	55.000**	<0.001
	Twice	16	26.2	13	14.1		
	3 times	13	21.3	22	23.9		
	4 or more times	-	-	52	56.5		
Does the child have trouble falling asleep?	Yes	52	54.2	79	82.3	*	<0.001
	No	44	45.8	17	17.7		
How long does it take the child to fall asleep?	Less than 15 minutes	24	25.0	17	20.7	22.349**	<0.001
	15-30 minutes	25	26.0	13	15.9		
	30-45 minutes	4	4.2	31	37.8		
	45-60 minutes	4	4.2	19	23.2		
	More than 60 minutes	-	-	2	2.4		
Does the child wake up tired in the morning?	Yes	12	12.5	58	60.4	*	<0.001
	No	84	87.5	36	38.3		
Does the mother have trouble awakening the child in the morning?	Yes	33	34.4	55	57.3	*	<0.001
	No	63	65.6	41	42.7		
How long does it take to awaken the child?	Less than 15 minutes	22	66.7	16	16.7	15.000**	<0.001
	15-30 minutes	9	27.3	34	35.4		
	30-45 minutes	2	6.1	6	6.3		
	45-60 minutes	-	-	2	2.1		
Does the mother think that the child has sleep problems?	Yes	35	36.5	78	81.3	*	<0.001
	No	61	63.5	18	18.8		

*Analysis was performed with the McNemar's test. **Analysis was performed with the Friedman test

Table V. Comparison of pre-post burn sleep habits

	Pre burn				Post burn				p value
	Never occasionally		Frequently every night		Never occasionally		Frequently every night		
The child;	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	
Has trouble falling asleep (needs a parent)	69	71.9	27	28.1	12	12.5	84	87.5	<0.001
Has trouble falling asleep at night again after waking up	67	69.8	29	30.2	16	16.7	80	83.3	<0.001
Wants a pacifier when he/she wakes up at night; wants his/her parent to put the pacifier back to his/her mouth	66	68.8	30	31.3	53	55.2	43	44.8	0.004
Wants to drink something overnight (Sucking Mother's Breast or bottle)	86	89.6	10	10.4	68	70.8	28	29.2	<0.001
Is afraid of sleeping alone	75	78.1	21	21.9	30	31.3	66	68.8	<0.001
Is afraid of sleeping in the dark	79	82.3	17	17.7	46	47.9	50	52.1	<0.001
Hugs an object while falling asleep (blanket, toy, bottle etc.)	70	72.9	26	27.1	71	74.0	25	26.0	>0.05
Is anxious and restless during the day because of not having enough sleep at the previous night	80	83.3	16	16.7	30	31.3	66	68.8	<0.001
Wants to be read books or listen to a lullaby before going to sleep	74	77.1	22	22.9	54	56.3	42	43.8	<0.001
Feels restless while sleeping	95	99.0	1	1.0	20	20.8	76	79.2	<0.001
Wakes up screaming, crying, wakes up due to a nightmare	95	99.0	1	1.0	23	24.0	73	76.0	<0.001

the number of the children who went to sleep between 9.00 p.m. and 10.00 p.m. at home decreased by 9.8% in the hospital, the number of children who went to sleep between 10.00 p.m. and 11.00 p.m. at home increased by 10.1% in the hospital.

In this study, the participating children went to bed later, had a greater number of sleep interruptions and woke up earlier in the morning in the hospital than they did at home. Similarly, in a study by Meltzer et al. (20), children went to bed later and had more sleep interruptions in the hospital than they did at home. However, in Meltzer et al. (20) study, the children woke up later and their total sleep time increased, which was different from the results of the present study. In Setoyama et al. (21) study conducted in 2016, the parameters such as durations of the whole-day sleep, time to go to bed, wake up time, time spent in bed, sleep efficiency, sleep onset latency, the number of night waking, duration of sleep after falling asleep in the hospital were compared with those at home, and significant differences were determined between duration of sleep at night and time they spent in bed, which was similar to the results of the present study. Although statistically insignificant, when they were in the hospital, the children woke up earlier, and the duration of daytime sleep and the total daily sleep times were longer.

In this study, 82.3% of the children had trouble falling asleep after suffering a burn, and it took 37% of them 30-45 minutes to fall asleep. Similarly, in Linder and Christian (22) study of hospitalized children, 60% of the children experienced delays in falling asleep although they had no trouble going to bed.

In this study, 36.5% of the mothers said that their children had sleep problems at home whereas 81.3% stated that their children had sleep problems in the hospital. Similarly, in their study conducted in 2014, Orme et al. (23) compared the children's sleep characteristics at home and in the hospital, and based on the parents' statements, they determined that the children's quality of sleep was better at home.

Study Limitations

In this study, the participants were not investigated in terms of the stages of sleep. In a study conducted by Armour et al. (9), they determined that thermal burns led to increases in sleeplessness and significant decreases in non-REM sleep (stage 3 and 4) and REM sleep.

The second limitation of this study was that sleep quality indicators such as fatigue etc. were not investigated by gender. In a study by Perdikaris et al. (24), they determined that fatigue levels of hospitalized children varied by gender, and that girls' fatigue scores were higher.

The third limitation was that the relationship between sleep quality parameters and the age of the children was not investigated in the present study. In a study conducted by Price et al. (25), the duration of daytime sleep and whole day sleep decreased as the children's age increased. The other limitation was that because the children's burn-

induced pain was intense in the first days post-burn, and because they were not expected to have overcome the burn shock immediately, the data were collected between the 2nd and 7th days of their stay in the hospital.

The last limitation was about the percentages of the burns. The number of patients with a percentage of burns greater than 20% during data collection was low. Different results may be obtained when studying patients with a higher percentage of burn.

Conclusion

In the current study, significant differences were determined between the participating children's pre- and post-burn conditions.

Burns cause major changes in the body metabolically. These changes generally show similar characteristics with the changes brought about by sleep deprivation. Nurses should be aware of the importance and benefits of sleep, should pay attention to sleep habits and characteristics of children they give healthcare to and make environmental arrangements to improve the quality of sleep in clinics in first week post-burn, which can minimize the triggering effect of staying in hospital on sleep disorders. It is recommended to carry out studies on the promotion of the sleep quality of hospitalized children and on changes in the effectiveness of Non-REM and REM sleep in children with burns.

Key Points

- There was a significant difference between pre-and post-burn periods on children's sleeping pattern.
- Children with a high degree of burn were shown to take longer to wake up in the morning.
- Supportive environmental regulations should be made to improve the quality of sleep in hospitals.

Ethics

Ethics Committee Approval: This study's procedure was approved by the Institutional Review Board of İzmir Katip Çelebi University Non-interventional Clinical Studies Institutional Review Board (approval number: 2015/112).

Informed Consent: A consent form was filled out by all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: H.Y.S., E.A.A., Design: H.Y.S., Data Collection or Processing: E.A.A., Analysis or Interpretation: H.Y.S., E.A.A., Literature Search: E.A.A., Writing: H.Y.S., E.A.A.

Conflict of Interest: No conflict of interest was declared by the authors.

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Original Article

Prevalence of Tonsillar Actinomycosis in Tonsillectomy Specimens; Clinical Importance and Management

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ABSTRACT

Aim: We aimed to assess the prevalence of actinomycosis in tonsillectomy specimens and its clinical importance and management in our center.

Materials and Methods: In this retrospective matched case-control study, patients who underwent tonsillectomy during 2010-2015 in Shahid Mostafa Khomeini Hospital, Tehran, were studied. Data regarding age, sex, indication for surgery (such as recurrent tonsillitis or sleep-disordered breathing), tonsil size, and length of disease before surgery were collected in both histopathologically positive (case) and negative (control) patients for actinomycosis.

Results: From the 260 studied tonsillectomy specimens, actinomycosis was found in 20 (8.75%) and 60 Actinomycosis negative cases were chosen randomly for the control group. There was no statistically significant difference between the groups with respect to age, sex, length of disease before surgery, and incidence of actinomycosis in tonsillectomy due to recurrent tonsillitis or sleep-disordered breathing. However, the tonsil size was significantly greater in specimens positive for actinomycosis (8.65±1.5 mL versus 4.38±0.22 mL, p<0.001).

Conclusion: Actinomycosis in tonsillectomy specimens is an incidental histopathological finding and does not necessarily correlate with active disease; while it is associated with tonsillar hypertrophy and increased tonsil size.

Keywords: Actinomycosis, tonsillectomy, recurrent tonsillitis, tonsillar hypertrophy

Introduction

Tonsillar Actinomycosis is a rare pathological condition in tonsillectomy specimens and its clinical importance is still controversial (1). Tonsillectomy is the most frequent surgery in children (2). The most common indications for tonsillectomy are recurrent tonsillitis and obstructive diseases such as sleep apnea, snoring, and mouth breathing (3). The histopathological examination of tonsillectomy specimens is done routinely to rule out occult malignancy or granulomatous disease (2-4).

The prevalence of *Actinomyces* in tonsil specimens varies from 1.3-37% (5-7). *Actinomyces* are a genus of gram-positive, pleomorphic, non-spore forming anaerobic, non-acid-fast branched filamentous bacteria (7). Six species of *Actinomyces* may cause disease in humans, but the most

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common isolated species are *Actinomyces israelii* and *Actinomyces naeslundii* (7).

The pathogenic *Actinomyces* species are commensals and saprophytic flora of the oropharynx, gastrointestinal tract and female genital tract and humans are a natural reservoir of pathogenic *Actinomyces* species (8).

Actinomycosis is a chronic disease which can form abscess, tissue fibrosis and secretory sinuses (9). The most common type for Actinomycosis is cervicofacial with a prevalence of 11-97% (10). The route of entry of the microorganism into the gastrointestinal tract from the mouth to rectum, is typically a break or trauma in the mucosa. Integrity of mucosal membrane is an important factor against infections. But this mechanical barrier is very thin and fragile in the gingival margin and tonsillar crypts and mucosal trauma in the tonsillar surface leads to a proliferation of these fastidious anaerobic bacteria in tonsillar crypts. *Actinomyces* species normally colonize tonsillar crypts and the oral cavity and many *Actinomyces* infections have odontogenic origins (9,10).

The prevalence of tonsillar Actinomycosis has been reported as higher in patients with sickle cell anemia, beta (β) thalassemia and β-hemolytic streptococcus (7). These conditions diminish the oxidation-reduction potential and cause a proliferation of *Actinomyces* in tonsillar tissue. This process may lead to tonsillar hypertrophy (7).

It has been suggested that tonsillar Actinomycosis may be an etiological factor in obstructive tonsillar hypertrophy and chronic tonsillitis (5,7,11-14). We aimed to assess the relationship between Actinomycosis in tonsillectomy specimens and the main causes of tonsillectomy as recurrent tonsillitis or sleep disordered breathing to calculate its prevalence in our center.

Materials and Methods

In this matched case-control study we gathered data from Actinomycosis histopathology samples of 20 patients who underwent tonsillectomy during 2010-2015 in Shahid Mostafa Khomeini Hospital, Tehran, Iran. To increase study power, we chose 60 Actinomycosis negative (An) tonsillectomy specimens for the control group. Power analysis shows that sample size was enough to assess associations.

Epidemiological data including age, sex, indication for tonsillectomy such as recurrent tonsillitis or sleep disordered breathing, tonsil size, and length of disease before surgery were recorded in both the Actinomycosis positive (Ap) and An groups.

Sleep disordered breathing was defined by snoring and mouth breathing during sleep or an irregular sleep pattern with clinically significant awakening from sleep with choking or gasping. Recurrent tonsillitis was diagnosed as greater than 6 acute tonsillitis attacks in 1 year or 3 in 2 consecutive years (2,3).

Tonsillectomy was performed with the cold dissection and suture ligation method and tonsils were sent in formalin to the pathology department. Histopathological analysis of the specimens was performed by two pathologists in the pathology ward of the hospital using direct microscopy and hematoxylin-eosin staining which is highly effective in showing *Actinomyces* colonies. Mean tonsillar size was recorded as the multiplication of length by height by width of tonsils measured and recorded in the pathologic results. The assessment of each tissue sample included an evaluation of mucosal surface, crypts and associated lymphoid tissue. The pathological feature of Actinomycosis was explained as aggregates of filamentous basophilic microorganisms arranged in radial spoke-like fusions. The diagnosis of Actinomycosis is established by two of the following conditions: positive culture, sulfur granules, and biopsy specimens showing the organisms (15).

Sulfur granules in infections other than Actinomycosis are very rare; therefore, their presence strongly supports the diagnosis (Figure 1). Although the ideal route for diagnosis is culture, its failure rate is very high because of the overgrowth of other microorganisms, lack of growth due to previous antibiotic therapy and inadequate anaerobic conditions or short-term incubation (16). Therefore, histopathological features from surgical specimens is the most preferred tool for diagnosis (Figures 2 and 3). Because our research was retrograde and on the pathologic specimens, and all the datas were without any information about patients,research was done completely secret and there was no need to ethics committee approval and informed consent.

Statistical Analysis

Logistic regression analysis was used to assess the relationship between tonsil size and Actinomycosis, after controlling confounding variables. Data were analyzed using SPSS software, version 16.

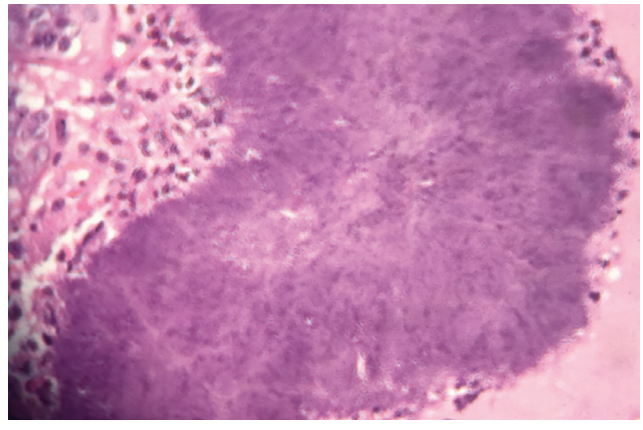

Figure 1. Sulfur granule

The categorical variables were analyzed with chi-square test and non-parametric data with Mann-Whitney U test.

Independent Variables: Data regarding age, sex, indication for surgery (such as recurrent tonsillitis or sleep-disordered breathing), tonsil size, and length of disease before surgery were collected.

Dependent Variables: Histopathologically positive (case) and negative (control) patients for actinomycosis.

Confounding Variables: Sex, indication for surgery (such as recurrent tonsillitis or sleep-disordered breathing)

Main Variable: Tonsil size.

Results

Twenty (8.75%) specimens (out of 260) were positive for Actinomycosis. Sixty (An) patients were chosen for the control group. The patients' demographic data are summarized in Table I.

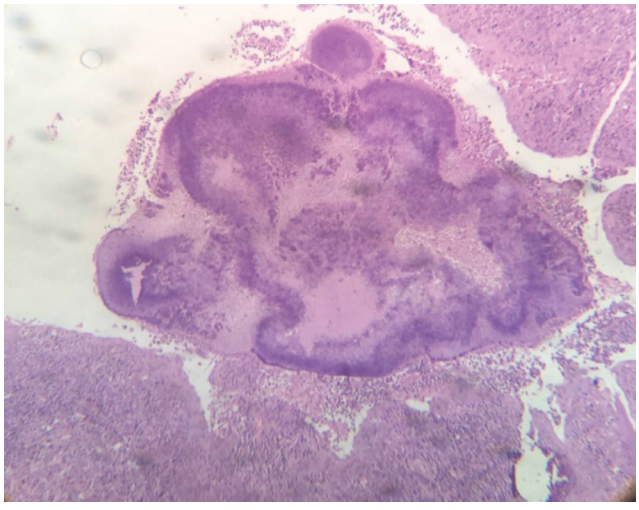

Figure 2. Actinomycosis clump formation

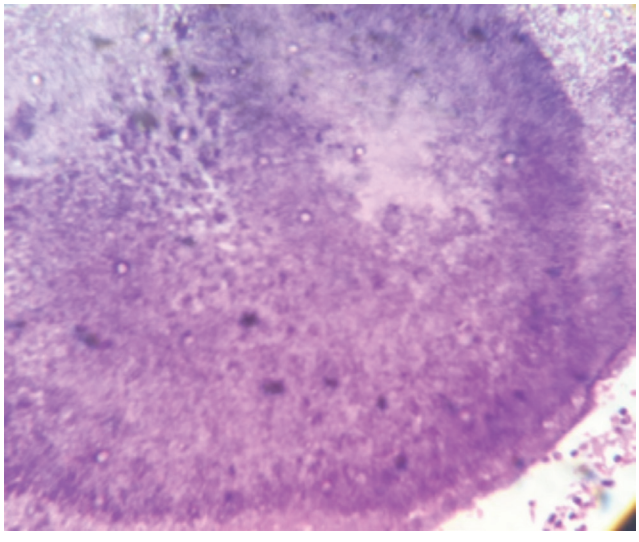

Figure 3. Aggregates of filamentous basophilic microorganisms in Actinomycosis

The mean ± standard deviation (SD) ages of patients in the Ap and An groups were 16.24±4.73 (range: 4-24 years) and 12.02±2.07 (range: 3-20) years, respectively (p=0.124). There were 9 (42.9%) women and 11 (57.1%) men in the Ap group and 26 (43.3%) women and 34 (56.7%) in the An group (p=0.927).

All the patients in the case group had recurrent tonsillitis and 14 (66.7%) had sleep disordered breathing as the indication for surgery. In the An group, 58 (98.3%) patients had recurrent tonsillitis and 31 (51.7%) had sleep disordered breathing. There was no statistically significant difference between the two groups in recurrent tonsillitis (p=1) or sleep disordered breathing (p=0.234).

Mean ± SD length of disease before surgery was 6.2±1 year in the Ap group and 5.8±1 year in the An group (p>0.05). The mean ± SD tonsil size in the Ap group was 8.65±1.54 mL and in the An group was 4.38±0.22 mL (p<0.001). Logistic regression analysis showed that Actinomycosis increased the tonsil size bz 1.8 times (OR: 1.8, CI: 95%, 1.2-4.3).

Discussion

Prevalence: In our study the prevalence of tonsillar actinomycosis was 8.75%. The prevalence of Actinomycosis in the tonsils varies from 1.3% to 37% (5-7) and it has been identified in 6.7% (17), 18.3% (5), 29.5% (20), 28.5% (7), and 11.5% (18) of all tonsil specimens in different studies. The wide range of variation in reported prequencies have been attributed to different staining techniques by different laboratories and a difference in patients and working groups as well as indications for tonsillectomy (17,18).

Age and Sex: Some studies reported a relationship between *Actinomyces* colonization and age (17-19). Ashraf and colleagues reported a mean age of 23.34 years for patients with actinomycosis and 15.91 years for those without actinomycosis indicating a positive correlation with older

Table I. Demographic data of the patients in the case and control groups

Demographic data	Actinomycosis positive	Actinomycosis negative	p value
Mean age (years)	16.24±4.73	12.02±2.07	>0.05
Sex	9 female, 11 male	26 female, 34 male	>0.05
Recurrent tonsillitis	20 (100%)	58 (98.3%)	>0.05
Sleep disordered breathing	14 (66.7%)	31 (51.7%)	>0.05
Duration of disease before surgery (years)	6.2±1	5.8±1	>0.05
Tonsil size (mL)	8.65±1.54	4.38±0.22	<0.001

age (19). Aydin et al. (17) and van Lierop et al. (18) and Toh et al. (6) and colleagues found that actinomycosis was more prevalent in adults than children. Some authors reported a female predominance of tonsillar Actinomycosis (1,17), while one study did not show such a predominance (20). We found no correlation between tonsillar actinomycosis and sex or age because of the low number of adults in the two groups.

Clinical Relationship: Several studies have aimed to identify the relationship between tonsillar Actinomycosis and clinical tonsillar disease including recurrent tonsillitis or sleep-disordered breathing (5,7,17,20,21).

This theory was suggested in 1910 by Lord stating that isolation of *Actinomyces* from tonsillar crypts may correlate with tonsillar hypertrophy due to the production of toxin by the microorganism (22). However, this has never been proven.

A higher prevalence of Actinomycosis was reported in specimens from tonsillectomy for obstructive symptoms compared with recurrent tonsillitis (5-7,14,23). They suggested that tissue colonization with *Actinomyces* may have an etiological role in lymphoid hyperplasia and tonsillar hypertrophy (5,7,14). Therefore, administration of a 12-week course of oral penicillin to all patients with obstructive symptoms has been suggested (5). However, it has not been proven by other studies (4,7,20,21). Toh et al. (6) demonstrated that Actinomycosis does not have any effect on tonsillar size while another study (23) reported larger sizes of tonsils with Actinomycosis. In our study, tonsillar size was significantly greater in the case group. Therefore, colonization of microorganisms may have an important role in size.

One study showed that the prevalence of *Actinomyces* colonization was higher in patients with recurrent tonsillitis than sleep-disordered breathing, but no statistically significant correlation was found between the presence of Actinomycosis and tonsillar hypertrophy or recurrent tonsillitis (19).

Another study found no relationship between clinical tonsillar disease and the presence of *Actinomyces*, in spite of finding a higher rate of cryptitis in tonsils with Actinomycosis, histopathologically (17).

A strong correlation was found between Actinomycosis and chronic tonsillitis showing positive tissue reaction to *Actinomyces* colonization in tonsil crypts suggesting its probable role in chronic and antibiotic resistant tonsillar symptoms (1).

In two other studies, no tissue reaction was found due to *Actinomyces* colonization in tonsils, therefore they reported no correlation between tonsillar Actinomycosis and recurrent tonsillitis (6,18). We also found no statistically significant difference in the indication for surgery such as recurrent tonsillitis or sleep disordered breathing between the two groups.

According to different studies it seems that *Actinomyces* colonization in tonsillar tissue does not indicate any active disease and there seems to be no correlation between its colonization and clinical tonsillar disease.

Histopathology: Tonsillar actinomycosis in the present study was diagnosed via the presence of sulfur granules in histopathological sectioning and confirmed by histopathologic analysis of specimens, but there was no specific tissue reaction such as cryptitis or micro-abscess formation or fibrosis in the specimens. The diagnosis of Actinomycosis was accurately done by the isolation of *Actinomyces* species from specimen culture or immuno-fluorescence staining, but less than 50% of cases were positive because of previous treatment with antibiotics, unsuitable culture media, or growth of other bacteria species (24,25). The presence of Actinomycotic sulfur granules in histological sections is highly supportive for diagnosis because these granules are very rare in other infections, however it is not diagnostic (26).

In one study, microorganisms were situated deeply in tonsillar crypts in histological examination but there was no specific tissue reaction to *Actinomyces* colonization (18). Gaffney et al. (20) found no relationship between the presence of Actinomycosis and tonsillar fibrosis or micro-abscess in reviewing the histology results of tonsil specimens with Actinomycosis. Aydin et al. (17) found cryptitis in tonsillar tissue and suggested that cryptitis can be used as a histological indicator of tonsillar Actinomycosis.

Riffat and Walker (27) used histopathological analysis with hematoxylin-eosin staining plus microbiological culture for Actinomycosis and suggested that *Actinomyces* colonization in tonsillar tissue cannot be neglected as passive saprophytes and its correlation with clinical diseases must be further studied.

Management: *Actinomyces* species are susceptible to several antibiotics such as penicillin G, erythromycin, and tetracyclines (8). Penicillin G is the drug of choice for all clinical forms of Actinomycosis and tetracycline is the drug of choice for patients who are allergic to penicillin (24,28).

The treatment of choice for Actinomycosis in chronic fibrosing conditions is long-term high doses of antibiotics to penetrate into the suppuration and fibrosis, in conjunction with drainage of the abscess and surgical excision of fistula and sinus tracts (24).

In some trials, oral penicillin for 12 weeks was suggested as a medical management of tonsillar Actinomycosis (5,29,30). However, in another study, this method appeared to be ineffective because, in some patients with sickle cell anemia who had received penicillin for a long period, there was Actinomycosis in their tonsils. Therefore, surgical excision has been recommended as the definitive treatment for head and neck Actinomycosis and tonsillectomy is the definite therapy for tonsillar Actinomycosis (7).

We hypothesis that the presence of *Actinomyces* in tonsillar specimens may have an etiological role in tonsillar hypertrophy and long-term tonsillar problems and chronic tonsillitis refractory to antibiotics. However, there was no specific evidence of tissue reaction to *Actinomyces* in tonsillar specimens and their presence was found to be due to superficial colonization of the tonsillar crypts. Therefore, it seems to be an incidental finding.

Study Limitations

This study was based on data gathered from patient medical records. We were not able to investigate the relation between sleep disorders and nasal obstruction. It is probable that some patients with history of mouth breathing suffered from a very common disease such as allergic rhinitis, which was not identified in patients with sleep-disorder breathing. This nasal obstruction may also affect our data analysis and influence some associations between sleep-disorder breathing and tonsil size.

Conclusion

In this study, we found no relationship between tonsillar Actinomycosis and tonsillectomy indications such as recurrent tonsillitis and sleep disordered breathing. However, tonsil size in Ap specimens were found to be significantly larger than An ones. Therefore, the presence of *Actinomyces* colonization in tonsillar specimens may have an etiological role in tonsillar hypertrophy and long-term tonsillar lesions and chronic tonsillitis refractory to antibiotics with no correlation or questionable relation with tonsillectomy indications such as recurrent tonsillitis and sleep-disordered breathing. However, this needs further study with a larger number of specimens or long-term prospective studies with meticulous methods for identifying the microorganism.

Acknowledgement

We are grateful for the collaboration of Miss Ghamzoda and Dr Elham Faghihzadeh for contributing in the statistical analysis. We also thank Dr. Mehdi Noroozi, epidemiologist and Dr Mohammadreza Jalali Nodushan, Pathologist, for their sincere support in data analysis, interpretation and photography.

Ethics

Ethics Committee Approval: Because our research was retrograde and on the pathologic specimens, and all the datas were without any information about patients,research was done completely secret and there was no need to ethics committee approval.

Informed Consent: Because our research was retrograde and on the pathologic specimens, and all the datas were without any information about patients,research was done completely secret and there was no need to informed consent.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: N.D., M.Y., P.I., Concept: P.I., Design: N.D., M.Y., P.I., Data Collection or Processing: N.D., M.Y., P.I., Analysis or Interpretation: N.D., M.Y., P.I., Literature Search: N.D., M.Y., P.I., Writing: N.D., M.Y., P.I.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Original Article

How Parents of Children with Cancer Seek Information Through Online Communities: A Netnography Study

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ABSTRACT

Aim: This study aims to examine how parents of children with cancer seek information through online communities, using netnography methods from qualitative study types.

Materials and Methods: The study was carried out through two online communities where parents of children with cancer in Turkey communicate with each other. The data were obtained from the parents' posts to online community platforms covering the period between September 2nd and November 2nd, 2017. The data were collected using the netnography method and analyzed within the principles of this method.

Results: A total of 580 written posts (information sharing) were sent within the interval in which the study was conducted, and 208 of them, which were posted by 131 parents, were analyzed and coded. Accordingly, three themes were defined in terms of codes as follows: "disease," "treatment and side effects", and "non-treatment" posts. The themes of "disease" and "treatment and side effects" included questions in which the parents were seeking information, whereas the theme of "non-treatment" mostly included posts in which the parents were seeking emotional support from other parents in the communities.

Conclusion: The way in which parents of children with cancer seek information through online communities is mostly related to their questions and posts parallel to the cancer process. In this process, the parents were found to seek emotional support.

Keywords: Children with cancer, parent, the internet, information seeking, netnography

Introduction

Pediatric cancers are increasing daily across the world (1). Pediatric cancer, which may occur in all ages of childhood, is a chronic disease requiring high-risk decisions depending on diagnosis and treatment (2). Therefore, it causes physical, psychological, social, and economic changes in the lives of both children and parents (1-5). Although parents receive supportive information and information about treatment from health professionals, they also seek more information from other sources about the cancer process (6). In today's modern world, the Internet has become a new information source for parents of children with cancer. Online communities also provide parents of children with cancer with an opportunity to create and use online social networks in order to gain information and support from people with similar experiences (7).

Health-focused online communities are defined as online platforms in which patients and families, who suffer

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from the same or similar diseases, share health information and experiences with each other and also provide social and emotional support to each other (7,8). There are many such platforms for parents of children with cancer. After their children are diagnosed with cancer, parents seek information and support (because of fear, anxiety, and not knowing what to do) (9). Many recent studies have drawn attention to parental online information-seeking behaviors and practices (2,6,9-18). Pehora et al. (10) report that Canadian parents actively use the internet to obtain child health information. DeLuca et al. (11) emphasize that parents of newborns mostly use the internet in search of newborn health information. Knapp et al. (12) point out that parents of children with life-threatening diseases use the internet substantially to find relevant medical information. Han and Belcher (9) report that parents of children with cancer use the internet to find cancer-related information, share experiences, and seek support. Gage and Panagakis (2) state that after children are diagnosed with cancer, parents start to use the internet mostly to get psychosocial support, rather than using it as a source of information about the disease. However, the number of studies on online information-seeking approaches adopted by parents of children with cancer is still very limited (2,9). This is the first netnography study conducted in Turkey to examine the information-seeking practices of parents of children with cancer via online communities. Using netnography methods, this study aimed to examine how parents of children with cancer seek information through online communities.

Materials and Methods

Netnography is defined as a new qualitative research methodology that adapts ethnographic research techniques to study cultures and communities that emerge through computer-mediated communication (19-21). Netnography is less intrusive and cheaper than other research methods (19,20). It follows certain methodological stages and directions: determining the research purpose, determining the appropriate online community, data collection, ensuring compliance with ethical standards, analysis and interpretation, and presentation of the research (19,22) (Figure 1). All the detailed features of the netnography method are described in a study by Kozinets (19). Ege University Health Sciences Scientific Research and Publication Board; 270-2017. Permissions of the moderators were obtained before the shares of the two network groups in this study were evaluated within the scope of the research.

Study Sample and Data Collection

First, an online community screening was performed using the Facebook search function and the keywords "cancer," "cancer and child," and "mother father" on the Internet. The Hope Foundation for Children with Cancer (KAÇUV), which has the highest number of foundation members in Turkey about 56.795 users (https://www.facebook.com/Kacuv/) was identified as the first online community for the study. Later, an online community of 30.055 users, called "dance with cancer" (https://www.facebook.com/groups/kanserledans/) was identified as the second online community for the study.

People can become members of the online communities KAÇUV and Dance with Cancer after their online membership requests are approved by the website moderators. These online communities have been established to support patients with cancer, and the rules to be followed by the members are monitored by the moderators. Dance with Cancer produces relevant content for all members from all age groups diagnosed with cancer. Members can send posts using their personal member accounts. Both online communities are specifically established for cancer patients and do not include medical advice.

Permission to collect data for our study using the online community platforms was obtained from the online community moderators. Dahan and Levi (23) emphasize that researchers should remain only as observers in netnography studies to collect robust data. Therefore, in the present study, the researchers only joined the online communities as members, and then collected the data objectively.

The shared content in this data included members' information-seeking and support-seeking approaches regarding cancer (questions, answers, and experiences). A total of 580 posts were sent in the study period between September 2 and November 2, 2017. Of these, 208 (about cancer, child, parent, or treatment) were included in the study. These posts were posted by 131 member parents who used the words "my child," "my son," or "my daughter." Posts sent by parents of children with cancer were included in this study, and those sent by adult cancer patients were excluded from the study.

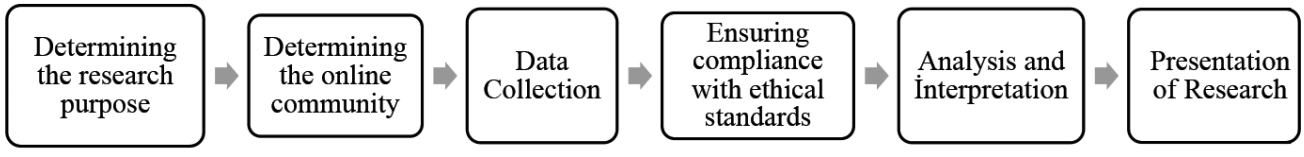

Figure 1. The methodological process of netnography
Kozinets R.V. The field behind the screen: Using netnography for marketing research in online communities. J. Marketing Res. (2002) 39:61-72.

The members were observed to use mostly colloquial speech and sincere expressions in their posts. However, there were many spelling and punctuation errors in their statements. The examples given in this study are presented without correcting the punctuation and spelling mistakes.

Statistical Analysis

The research analysis was carried out in accordance with the principles of coding and analysis adapted from the netnography method of Kozinets (19). The data were evaluated by two researchers. After reading all posts in the sample more than once, the researchers identified three main themes: (1) "disease" posts, (2) "treatment and side-effects" posts, and (3) "non-treatment" posts. Based on these themes, descriptive encodings of the posts were made until a new theme did not occur. After the codes were discussed and finalized by two researchers, they were transferred to the MAXQDA Analytic Pro 12 (release 12.3.1) program for analysis.

Results

The research results are discussed under two headings: "gender distribution" and "themes."

Gender Distribution

Parents emphasized their parental identities, using the expressions "my son," "my daughter" and "my child." Parents included 16 fathers and 92 mothers. However, 23 of the parents defined themselves only using the word "parent's" and did not specify their identities as mother or father (Table I). This study uses the word "parents" referring to all mothers and fathers whose posts were included in the study.

Themes

Three important themes were determined regarding the parents' information-seeking through online communities: "disease," "treatment and side effects" and "non-treatment" posts. The distribution of themes is shown in Figure 2 (MAXQDA Analytic Pro 12).

Table I. Gender distribution in online communities

Parents of children with cancer (n=131)	n	%
Mother	92	70
Father	16	12
Unspecified parent*	23	18

*These are parents who did not state whether they were mothers or fathers

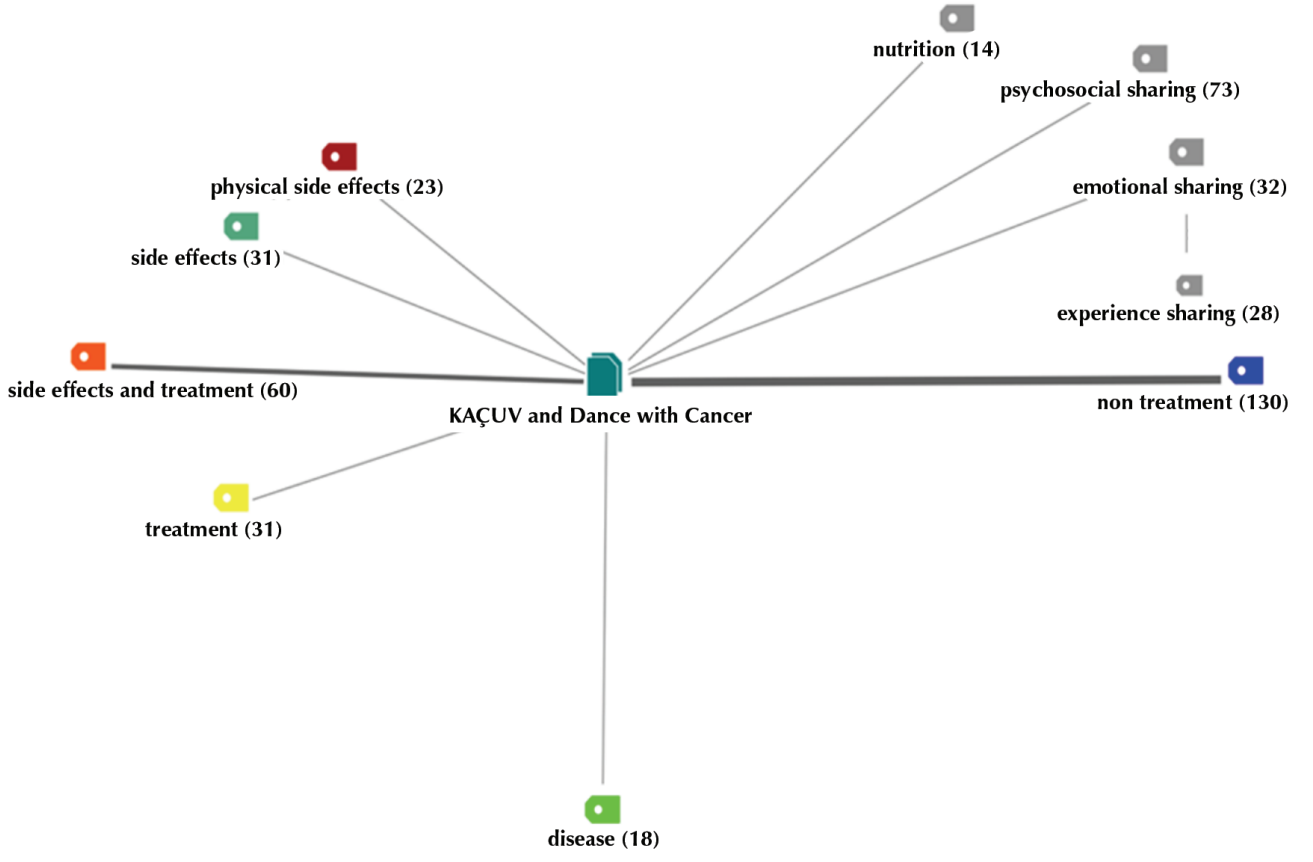

Figure 2. Distribution of the most posted sub-codes

1. Disease Posts

The parents asked questions that reflected their information-seeking about the disease (n=18). Some of the posts in this theme are listed below:

" .... what kind of a path is hepatoblastoma disease following?," "....we are currently waiting for the nerves to heal, magnetic resonance results are clean, will the treatment continue?" "what is the percentage of relapse in acute lymphoblastic leukemia after the transplant?" and "what is the rate of secondary cancer?."

2. Posts about Treatment and Side Effects

The "treatment and side effects" posts were high in number (n=60). The parents had clear questions:

- "We are in the first month of my son's leukemia treatment, what will happen?" and "My child frequently receives radiation therapy and magnetic resonance, how long will this continue?"

One of the parents directed his/her information-seeking questions about treatment to other parents, and he/she was found to get answers containing emotional support statements:

- "Is there anyone who has undergone autologous tissue transplantation due to leukemia, if so, could you please write to me?" "Get well soon! My daughter underwent marrow transplant surgery when she was 4 years old, it was one year ago, Thank God! We are OK now" "my daughter underwent the surgery on 24th of October the last year, we used femodal pills, she received radiotherapy for 30 days and had 8 chemotherapy sessions. Anaplastic ependymoma grade 3. She is 4-5 years old now, the cancer was in her brain stem. I hope my little baby survives."

The "physical side effects of chemotherapy/radiotherapy" posts (n=23) were interesting. The most commonly mentioned side effects were fever, mucositis, nausea, loss of power, malaise, hair loss, and gait. In addition, there were information-seeking posts regarding side effects such as cough, organ damage, muscle laziness, growth retardation, pain, and numbness in the hands (Figure 3, MAXQDA Analytic Pro 12):

- "What is the cause of fatigue after radiotherapy?," "The hearing loss has started, so what should we do?," "my child doesn't want to eat, saying (he/she has) nausea, what method should I follow?," "... the nausea does not disappear despite the drugs," "... how should I talk to my child? how should I tell my child to eat?," "... how to get rid of the pain of mouth sores caused by chemotherapy?," "My child is recovering from the medication that he receives for mouth sores, but after a short time these sores reappear, and my child is having trouble even in drinking water. What should

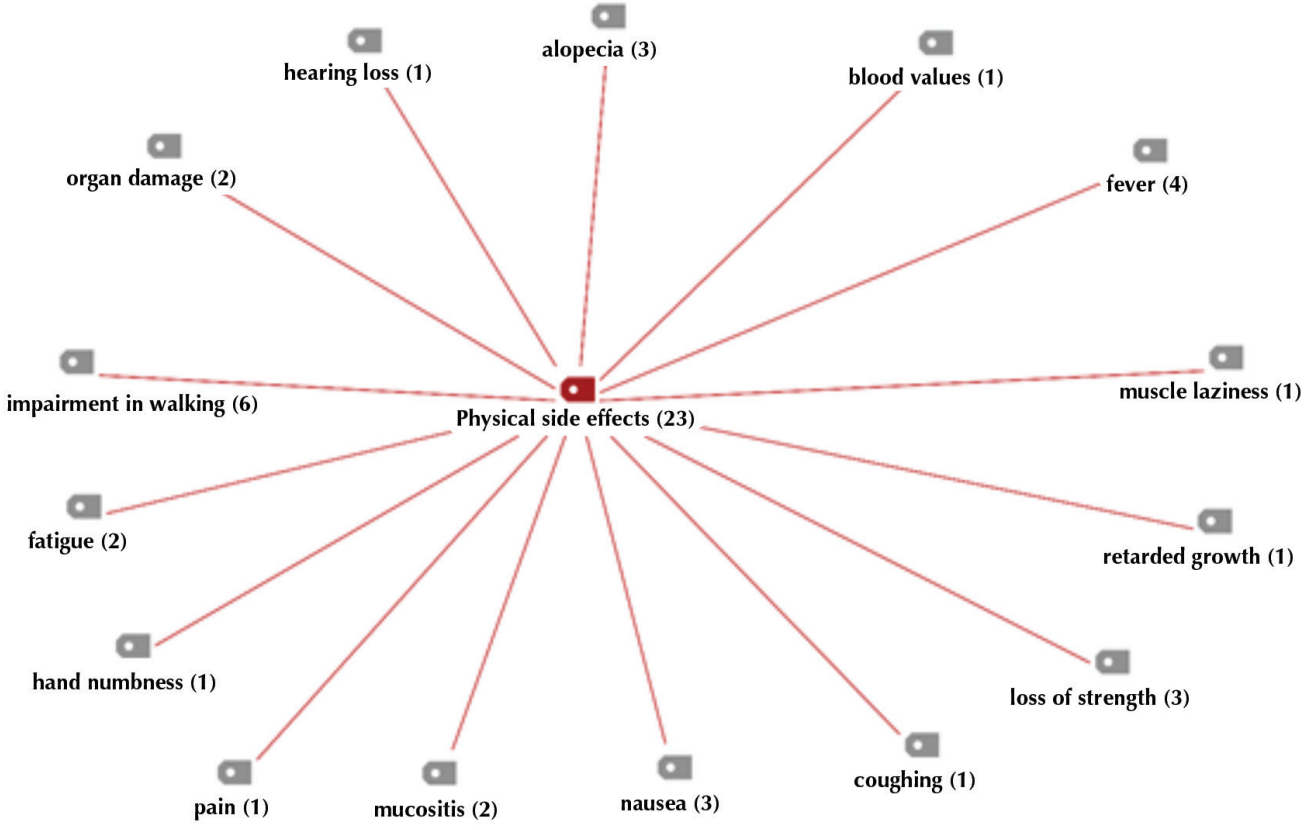

Figure 3. Distributions of the sub-codes regarding physical side effects of chemotherapy/radiotherapy

I do?" "My child doesn't want to eat, saying his stomach is sick, how should I proceed?" "The nausea still continues despite the drugs we used."

One of the parents sought information in her post, but also referred to the traumatic feeling that the side effect posed for her child: "The children do not cry because of the disease, but they are very upset because of their hair loss" and "Finally she could fasten hair-clips to her hair, and now she has ponytails."

3. Non-treatment Posts

The non-treatment themes made up the most heavily used area (n=130), and included posts about psychosocial conditions (n=73), emotional expressions (n=32), experience-sharing (n=28), and nutrition (n=14).

"Non-treatment" posts constituted the most emotionally intense category, as these posts included discussions about parent experiences, treatment results, parental expectations, and parents' support-seeking expressions. Some remarkable posts with supportive and promising emotional expressions were as follows.

- "My child struggled with the same disease, now (she is) 3.5 years old, we're good now, we have recovered from the disease," "Your greatest medication is morale and motivation... Never think badly," "I hope you get good news when your treatment is over," "Thank God! We're all good, we will overcome it (the disease) ..."

One of the parents explained that these supportive expressions had a positive impact on her:

- "My dear friends, I have read through my old posts, I was moved to tears. Sometimes, I asked some questions for my daughter suffering from neuroblastoma, sometimes I asked for prayers, sometimes I shared that the chemotherapy wasn't working, and you gave me support, I shared with you that my daughter's imaging was clear and you were happy for me, this was so precious to me, most of the time I was able to stand strong with the support of my friends."

Apart from the questions about treatment and its side effects, parents asked remarkable questions about nutrition for their children and searched for information: "what can we eat?" "Are cola or chips forbidden?," "Is it enough to keep the vegetables in vinegar for 20 minutes?"

Discussion

This study was conducted to examine how parents of children with cancer seek information through online communities. In addition, it is the first netnography study conducted in Turkey to examine information-seeking practices of parents of children with cancer via online communities. The majority of those who sent posts were mothers with caregiver roles. Gage and Panagakis (2) and Han and Belcher (9) conducted a study of parents of children with cancer and reported that it was mostly mothers who cared for their children, so mothers posted a higher number of posts to the internet than fathers did. Plantin and Daneback (18) examined the information-seeking practices of parents of children who were patients and observed that mothers constituted the majority of parents actively using the internet. In general, mothers take an active caregiver role for their children with cancer, because cancer is a chronic disease, the treatment is challenging, and it has side effects reducing the quality of life.

The cancer treatments that begin immediately after diagnosis of the disease include surgery, chemotherapy, radiotherapy, biotherapy, and stem cell transplantation (1,3). Parents communicate with many healthcare professionals during and after this complex cancer treatment. In addition, parents become decision makers in the treatment process by undertaking the primary caregiver role for their children with cancer (24). Therefore, they are responsible for closely monitoring the health and treatment of their children (24,25).

In our study, parents were in search of information about cancer, its treatment, and side effects. In addition, they were observed to look for non-treatment-related support. These results were consistent with parents' information-seeking and support-seeking behaviors observed in similar studies (2,6,9-18).

In addition to their own information-seeking about the "disease" and its "treatment and side effects," the parents were observed to seek information about what other parents with similar health problems do and know. Yeh et al. (26) report that parents make a lot of effort to obtain information about their child's disease and exchange information with other parents to manage the disease's progress and side effects. In fact, treatment-related side effects of pediatric cancers are difficult to assess and take a long time to manage (3,5). Henström et al. (27) emphasize that cancer treatment is a very traumatic process. Similarly, in our study, the parents also emphasized the traumatic aspect of cancer treatment. In a study conducted by Hildenbrand et al. (28), one of the parents involved in the study had stated, referring to her child, that the most difficult part for her was the treatment process. From the posts, parents were observed to undertake the primary care of their children in the treatment process and in the accompanying side effects, to follow the treatment methods and procedure, and to seek relevant information. Wainstein et al. (6) and Tuffrey and Finlay (16) report that the majority of parents use the internet to search for more information about the disease and treatment. DeLuca et al. (11) point out that parents share the questions of "what?" "What happens," "How will it be treated?" and "Is it treatable?" while seeking information about the disease. As can be seen, the parents' information-seeking practices and

behaviors in this study are similar to those observed in the literature. Gage and Panagakis (2) emphasize that the internet is a secondary information source in the online information-seeking practices used by parents of children with cancer. They also report that parents of children with cancer send posts regarding the basic information about cancer and its treatment, using such questions as "What is transplantation?" and "How is it done?" which are similar to the questions posted by the parents in our study.

Children with cancer are always at risk of dying (1,4). The parents exhibited their information-seeking practices by sending posts about how they felt about this possibility and how to deal with it. In cancer, the treatment process not only affects the quality of life of those children with cancer and their parents (29), but also leads to anxiety and depression in the parents (26,30,31). Therefore, in the present study, the parents also wanted to have information about the disease and sought emotional support.

In the "non-treatment" posts, the parents were observed to seek emotional support. The parents used supportive and promising emotional expressions in the posts they sent to other parents whose children had suffered from the same health problems. Studies indicate that parents need emotional support during the treatment process and get support from other parents' posts, including statements of hope, prayer and good wishes (2,9). In our study, by sending posts like "Thank God! We are well now, we will recover" and "I hope we will get good news", the parents also expected other parents to send them posts including statements of hope-prayer-good wishes. In a study conducted by Han and Belcher (9), one of the parents of a child with cancer said that "some people in the community were very inspiring. I felt I was growing spiritually and emotionally, just by reading their writings", which indicates that the parents seek both information and support from online communities and share their feelings and emotional states through these communities. Similarly, in our study, parents were observed not only to seek information about the disease of their children, but also to seek emotional support from other parents in similar situations. This emotional support positively affects them. Studies show that sharing supportive expressions and experiences on the internet positively affects the quality of life of parents of children with cancer (2,9).

The parents' information-seeking behaviors indicate that they have parental potential with a tendency to wonder, want to learn, and share. Parents clearly and sincerely expressed their feelings, sorrows, and expectations, as well as their need for informational and emotional support regarding the difficulties they experienced with regards to their children.

In this study, another point outside the themes was noticed when the parents' posts in the two online communities were examined: there was no information provided by an oncologist or pediatric oncologist. This is a highly significant situation, because health professionals (oncologists, pediatric oncology nurses, etc.) can provide accurate and safe information. Pehora et al. (10) pointed out the need for health professionals to play a role in improving accurate and reliable information access on the internet. Another study emphasized that oncologists and pediatric oncology nurses can provide parents of children with cancer with reliable information on the internet (9). It will be meaningful to include this issue in new studies.

Study Limitations

Netnography, a qualitative research type, was used in this study. The posts of parents in only two online communities were included in the study, which is an important study limitation. Another study limitation is that the data were collected over a very limited period of time. These qualitative data were descriptive and exploratory; they were not designed for formal hypothesis testing. In addition, although internet use is widespread nowadays, the possibility of existing parents who cannot reach the Internet constitutes another study limitation.

Conclusion

Parents of children with cancer seek information through online communities, mostly searching for information about cancer, its treatment, and side effects. In addition, parents also seek emotional support from parents in similar situations in these online communities. The parents provide emotional support to each other. There was no information from an oncologist or from a pediatric oncology nurse among the posts sent through the online community platforms in our study. Therefore, online pediatric oncology specialists and pediatric oncology nurses and health professionals should be made aware of online communities so they can provide reliable information and professional support in these areas.

Ethics

Ethics Committee Approval: Ege University Health Sciences Scientific Research and Publication Board; 270-2017.

Informed Consent: Permissions of the moderators were obtained before the shares of the two network groups in this study were evaluated within the scope of the research. Personal privacy and parental nicknames were not shared.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: C.S.G., S.Ş., Design: S.Ş., C.S.G., Data Collection or Processing: C.S.G., Analysis or Interpretation: C.S.G., S.Ş., Literature Search: C.S.G., S.Ş., Writing: S.Ş., C.S.G.

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Original Article

Investigations of Microtubule-associated Protein 2 Gene Expression in Spinal Muscular Atrophy

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ABSTRACT

Aim: Spinal muscular atrophy (SMA) is a devastating genetic disease in childhood andff is caused by the absence of functional survival motor neuron (SMN) protein, which leads to impairments of the cytoskeleton, especially in neurons. Dysregulation of actin dynamics have been linked to SMA patho mechanisms, however involvement of altered microtubule dynamics is largely unknown. In this study, we investigated differentially expressed microtubule-related genes using *in vitro* and *in vivo* SMA model systems.

Materials and Methods: By focusing on microtubule-related genes, we re-analyzed publically available gene expression arrays, which were previously performed with induced pluripotent stem cell-derived motor neurons of SMA patients and the spinal cords of SMA mice. We found altered expressions of microtubule-associated protein 2 (MAP2), which was validated by real time reverse-transcription polymerase chain reaction using the SMN knock-down NSC34 cell line and the severe SMA mouse model.

Results: We showed that the expression of *MAP2* gene was significantly upregulated in both expression arrays. Upregulation was also detected in the brain and spinal cord tissues of severe SMA mice at different developmental stages.

Conclusion: Our findings suggest that microtubule regulatory proteins may be altered in SMN depleted cells and further research is needed to elucidate the contribution of dysregulated microtubule dynamics towards SMA.

Keywords: Spinal muscular atrophy, exon-array, microtubule-associated protein 2

Introduction

Spinal muscular atrophy (SMA) is an inherited neurodegenerative/neuromuscular disease and the leading genetic cause of infant mortality. The incidence of SMA is reported as 1 in 11.000 live births, however, due to a high rate of consanguinity, it is estimated to be higher in Turkey (1). SMA is characterized by the loss of alpha motor neurons in the spinal cord and progressive muscle atrophy. Since patients have different clinical phenotypes, SMA is grouped into V Types (0-IV) according to the age of disease onset and achieved motor functions (2,3). Type 0 refers to the most severe and the Type IV refers to the mildest form of SMA. Mutations or conversions of the *Survival of motor neuron 1 (SMN1)* gene are responsible for SMA and regardless of disease severity, homozygous deletion of exon 7 and 8 or exon 7 only is the most frequent mutation in patients (4-6). It has been shown that the absence of ubiquitously expressed, functional SMN protein leads to defects in both axon and dendrite growth, axonal transport and neuromuscular junction

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maturation in model systems and also patient samples (7-11). Dysregulation of F-actin dynamics have been linked to these defects due to alterations in either actin-regulatory proteins such as profilin, plastin 3, coronin 1C or Rho-kinase (ROCK) signaling pathways in SMN depleted cells (6,7,12,13). Although significant alterations in some microtubule-related proteins (stathmin and tau) have been shown, the contribution of altered microtubule dynamics to SMA patho mechanisms is largely unknown (14,15).

Re-analysis of publicly available gene expression data is a powerful and cost-efficient technique to better understand disease mechanisms. This technique has been used to explore the molecular mechanisms of various diseases, such as different cancers (16,17), osteoarthritis (18) and degenerative diseases (19). Furthermore, meta-analysis and the comparison of gene expression profiles of different species enables researchers to discover conserved molecular mechanisms (20).

Therefore, in this study, we re-analyzed human and mouse microarray gene expression data and specifically focused on genes regulating microtubule structure and function. We found that the expression of MAP2 was significantly altered in both induced pluripotent stem cell (iPSC) derived motor neurons of SMA patient and the spinal cords of SMA mice when compared to controls. MAP2 is primarily expressed in neurons and localizes to cell bodies and dendrites in mature neurons. The MAP2 protein binds to microtubules and regulates their stability. It also binds to F-actin and bundle filaments *in vitro* (21). Therefore, we focused on the *MAP2* gene and analyzed its expression in both the SMN knock-down motor neuron like NSC34 cell line and the Taiwanese SMA mouse model (22).

Materials and Methods

Human and Mouse Dataset Retrieval

The Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/, (23,24) database was searched for Human Exon arrays of motor neuron samples obtained from an SMA patient and a control. A record GSE27205 (25), which was on the Affymetrix Human Exon 1.0 ST platform (HuEx-1_0-st), was identified and CEL files of iPSC-derived motor neurons from an SMA patient (n=3, n is different clones from an SMA patient; GSM672172, GSM672173 and GSM672174) and a heterozygous father (n=3, n is different clones from an SMA patient's father; GSM672178, GSM672179 and GSM672180) were selected and extracted from the GEO database. This article does not contain any studies with human participants performed by the authors. Informed consent wasn't obtained. Mouse microarray data GSE19674, which was on an Affymetrix Mouse Genome 430A 2.0 Array platform (Mouse430A_2), including CEL files of spinal cords of homozygous knock-out SMA mice (n=4, $SMN2^{+/+}$; SMN $\Delta7^{+/+}$; $mSmn^{-/-}$; FVB.Cg-Tg (SMN2*delta7) 4299Ahmb Tg (SMN2) 89Ahmb $Smn1^{tm1Msd}$) and heterozygous SMN knock-out mice (n=4, $SMN2^{+/+}$; $Smn\Delta7^{+/+}$; $mSmn^{+/-}$; FVB.Cg-Tg (SMN2*delta7) 4299Ahmb Tg (SMN2) 89Ahmb $Smn1^{tm1Msd}$) (26). CEL files for SMA mice spinal cord (GSM491297, GSM491298, GSM491299 and GSM491300) and heterozygous SMN knock-out mice spinal cord (GSM491293, GSM491294, GSM491295 and GSM491296) were obtained from the GEO database.

Human and Mouse Gene Expression Analysis

Affymetrix Human Exon 1.0 ST array CEL files of an SMA patient and heterozygous father, and Affymetrix Mouse Genome 430A 2.0 Array CEL files of an SMA and heterozygous SMN knock-out mouse were analyzed via the Transcriptome Analysis Console 4.0.1.36 (TAC, https://www.thermofisher.com). For the human exon array analysis, Gene Level-Core, robust multichip average (RMA)-sketch workflow was applied to create probe level summarization files. For Mouse430A_2 arrays, the RMA algorithm (27,28) was used to normalize CEL files. The annotation files HuEx-1_0-st-v2.na36.hg19.transcript.csv for HuEx-1_0-st array and Mouse430A_2.na36.annot.csv for Mouse430A_2 array were used to annotate human and mouse arrays, respectively. Probe sets with ANOVA (eBayes) p-value <0.05 and fold change <-1.5 or fold change >1.5 were considered as differentially expressed for both human and mouse datasets.

Venn Diagram Analysis

The significantly altered genes, which are involved in microtubule structure and regulation of its dynamics, of human and mouse datasets were compared using Venny 2.1 (http://bioinfogp.cnb.csic.es/tools/venny/) (29).

Cell Culture and siRNA Transfections

Motor neuron-like murine NSC34 cells were grown in Dulbecco's modified Eagle medium (DMEM, 4.5 g/D-glucose), containing 5% fetal calf serum and 1% penicillin/streptomycin at 37 °C, 5% CO_2. The cells were transfected with siRNA against murine SMN (5'-CAGAAGUAAAGCACACAGCAA-3') or scrambled control siRNA (5'-GCGCAAAUAAACCGAAAGACA-3') by using OptiMeM (Thermo Scientific) and Lipofectamine 2000 (Invitrogen) in differentiation medium, containing 1% FSC for 72 hours. The SMN knock down efficiency of NSC34 cells was about 80%, which was routinely tested by Western blot.

RNA Isolation and Real Time RT-PCR

Total RNA was isolated from NSC34 cells by the RNeasy mini kit (Qiagen) using the manufacturer's protocol. Spinal

cord (p1, p5 and p8) and brain (p8) RNA samples of severe the SMA Taiwanese mice model (FVB.Cg-Tg (SMN2) 2Hung SMN1^{tm1Hung}/J (22) (Jackson Laboratory) and heterozygous control littermates, which were previously isolated and stored at -80 °C, according to German animal welfare regulations (breeding approved by the Lower Saxony State Office for Consumer Protection and Food Safety (LAVES, reference number 15/1774 LAVES). The numbers of mice used from different developmental stages are provided in the figure legends. cDNA synthesis was performed as previously reported (30). Briefly, 2.5µg of RNA was incubated with random hexamer primers (3µg/µl, Invitrogen) at 70 °C for 2 min, then M-MLV-transcriptase (200U/µl, Invitrogen), RNase-Inhibitor (40U/µl), DTT (0.1M, Invitrogen) and dNTP (10mM) was added. The reaction mix was incubated at 42 °C for 90 min and then 70 °C for 15 min for transcriptase deactivation. Real-time reverse-transcription polymerase chain reaction (RT-PCR) was performed using 5µl diluted cDNAs (1:200), 7µl SYBR green (Applied Biosystems, PowerSYBR green mix), 2µl MAP2 primers (1.75µM, forward: 5'TCTAAAGAACATCCGTCACAGG3', reverse: 5'GGTGAGCATTGTCAAGTGAGC 3') or PPIA primers (1.75µM, forward: 5'TGCACTGCCAAGACTGAATG 3', reverse: 5'CCATGGCTTCCACAATGTTC 3') as the housekeeping gene. The reaction was performed in triplicate and StepOnePlus Real-time PCR System (Applied Biosystems) was used with the following conditions; 95 °C for 10 min (initial denaturation), 40 cycles at 95 °C for 15 sec and 60 °C for 1 min. A comparative threshold cycle method (2-$^{\Delta\Delta CT}$) was used for the quantitation of the results.

Statistical Analysis

Statistical analyses were performed using Graphpad prism version 8 (La Jolla California USA). Mann-Whitney U test was used and a result of $p<0.05$ was considered as statistically significant.

Results

After the re-analysis of human and mouse array CEL files, we identified differentially expressed genes of SMA samples when compared to their related control samples in both human and mouse datasets (ANOVA (eBayes) p-value <0.05 and fold change <-1.5 or fold change >1.5). In order to find expressional alterations of microtubule-related genes in SMA patients and SMA mice, we analyzed the expression of several transcripts, which are involved in both microtubule structure and the regulation of its dynamics. Differentially expressed gene lists of human and mouse datasets are given in Table I and II, respectively.

Among the microtubule-related genes that we analyzed in this study, 5 genes were differentially expressed in both the SMA patient and mouse model, while MAP2, MAP7 and TUBB4A showed similar gene expression alteration patterns (Figure 1A). The *MAP2* gene was the only upregulated target, which drew our attention since the altered protein level of MAP2 was reported in a mouse model of amyotrophic lateral sclerosis (ALS), which is another motor neuron disease (31). Additionally, it has also been reported that either the protein level or the post-translational modifications of TAU, which is another microtubule-associated protein from the same protein family, was altered in ALS and SMA models, respectively (14,21,31). Therefore, we subsequently focused on the *MAP2* gene. To validate exon array results, we first used motor neuron-like NSC34 cells, which are murine neuroblastoma and spinal cord hybrid cell line as an *in vitro* model (32). We knocked down SMN by siRNA in the NSC34 cells and detected an upregulation in *MAP2* gene expression by real time RT-PCR in SMN-depleted cells compared to scrambled controls (Figure 1B). Since the increase in gene expression was close to the significance level, we decided to analyze *MAP2* gene expression in the severe SMA Taiwanese mouse model (22). First, we analyzed *MAP2* gene expression in the total brain and spinal cord of late symptomatic p8 mice. We found a significant upregulation in both tissues of SMA mice compared to control littermates (Figure 1C). Considering that spinal cord motor neurons are primarily affected by SMN loss, we further analyzed *MAP2* gene expression in the spinal cords of both pre-symptomatic (p1) and early symptomatic (p5) mice. A significant increase was detected in p5 but not p1 SMA mice (Figure 1C).

Discussion

SMA is a devastating genetic disease of childhood. Despite the recent therapeutic achievements with antisense oligonucleotide and successful clinical trials with gene therapy and small molecules, elucidating the functions of SMN protein and understanding the patho mechanisms of SMA is still needed. Re-analysis of public gene expression data is a promising tool to understand disease mechanisms. In this study, we re-analyzed raw data of previously published human exon-array and mouse microarray data that we obtained from the GEO database (23,24) and specifically focused on genes regulating microtubule structure and/or function, since there is little knowledge about microtubule dynamics in SMA. Previously, an altered organization of microtubules in the presynaptic terminals of the axons innervating transverse abdominis of SMA mice have been reported in this commonly affected muscle (33). Additionally, stathmin, a microtubule depolymerizing protein, is upregulated in both SMN-depleted NSC34 cells and SMA mice and has

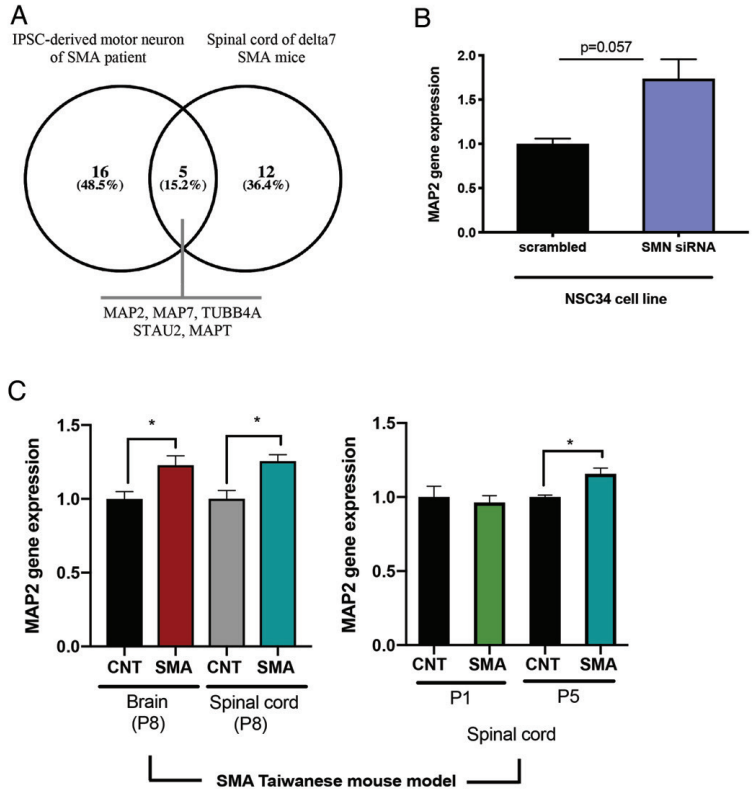

Figure 1. Expression analysis of genes regulating microtubule structure and its dynamics, A) Venn diagram of significantly altered genes in induced pluripotent stem cell-derived motor neurons of SMA patient and delta7 SMA mice using HuEx-1_0-st and Mouse430A_2 datasets, respectively. Fold changes of MAP2 transcript level in B) SMN knock down NSC34 cell line, n=4 biological replicates, C) spinal cord and brain tissues of p1, p5 and p8 severe SMA Taiwanese mouse model and heterozygous control littermates, for p1; n=5 (control) and n=4 (SMA) mice, for p5; n=6 (control) and n=5 (SMA) mice, for p8; n=5 (control) and n=5 (SMA) mice. PPIA gene was used for normalization. Mann-Whitney U, *p<0.05, Data are presented as means with standard error of mean.

MAP: Microtubule-associated protein, SMN: Survival of motor neuron, SMA: Spinal muscular atrophy

Table I. Expressional alterations of microtubule-related genes in iPSC-derived motor neurons of SMA patient

ID	Fold change	p-value	FDR p-value	Gene symbol	Description
*3591459	2.24	0.0426	0.1914	MAP1A; KRTAP6-1	microtubule associated protein 1A; keratin associated protein 6-1
3860229	1.71	0.001	0.0441	CLIP3	CAP-GLY domain containing linker protein 3
2790823	1.61	0.021	0.1322	MAP9	microtubule-associated protein 9
*3784208	1.59	0.0151	0.1103	DTNA; MAPRE2	dystrobrevin, alpha; microtubule-associated protein, RP/EB family, member 2
2525533	1.56	0.006	0.0718	MAP2	microtubule associated protein 2
3740126	-1.5	0.0472	0.2023	YWHAE; PAFAH1B1	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon; platelet-activating factor acetylhydrolase 1b, regulatory subunit 1 (45kDa)
3526151	-1.56	0.0258	0.1464	TUBGCP3	tubulin, gamma complex associated protein 3
4002081	-1.62	0.0034	0.0575	MAP7D2	MAP7 domain containing 2
2654394	-1.68	0.0467	0.2014	FXR1	fragile X mental retardation, autosomal homolog 1
3721926	-1.73	0.0046	0.0642	TUBG1	tubulin, gamma 1
3140640	-1.75	0.0081	0.0823	STAU2	staufen double-stranded RNA binding protein 2

Table I. Continued

ID	Fold change	p-value	FDR p-value	Gene symbol	Description
*3723687	-1.87	0.0026	0.0524	MAPT; MAPT-IT1	microtubule associated protein tau; MAPT intronic transcript 1
2901913	-1.88	0.0022	0.0504	TUBB	tubulin, beta class I
3764933	-2.03	0.0193	0.1264	TUBD1	tubulin, delta 1
*4050485	-2.05	0.0116	0.0968	GRIN1; TUBB4B	glutamate receptor, ionotropic, N-methyl D-aspartate 1; tubulin, beta 4B class IVb
3847959	-2.24	0.0012	0.0443	TUBB4A	tubulin, beta 4A class IVa
2600068	-2.27	0.0026	0.0524	TUBA4A	tubulin, alpha 4a
2878662	-2.51	0.0045	0.064	DIAPH1	diaphanous-related formin 1
3515965	-2.73	0.0071	0.0777	DIAPH3	diaphanous-related formin 3
2975741	-2.81	0.0002	0.0436	MAP7	microtubule-associated protein 7
*3453732	-16.34	0.0447	0.1965	TUBA1B; LMBR1L; TUBA1A	tubulin, alpha 1b; limb development membrane protein 1-like; tubulin, alpha 1a

*is used where the probe group is annotated to different gene symbols

been linked to defective microtubule polymerization (15). Hyperphosphorylation of TAU protein has been reported in the spinal cord of both SMA mice and patient samples (15). We observed an opposite gene expression profile of microtubule associated protein TAU (MAPT) between human and mouse gene expression results. According to our analysis, this target showed downregulation in human iPSC-derived motoneurons but it was upregulated in the spinal cords of SMA mice, which might be related to the presence of glial cells in the spinal cord (Table I and Table II). We focused on genes which have similar expression pattern in both arrays such as MAP2, MAP7 and TUBB4A. Among them, the *MAP2* gene was the only upregulated one in both SMA patient and SMA delta 7 mice. It has been known that MAP2 plays a role on neuronal growth and degeneration (34). Considering its function in regulating microtubule stability in neurons and previous reports on gene expression alterations in ALS, we analyzed *MAP2* gene expression for both *in vitro* and *in vivo* SMA model systems. Our experimental results were consistent with mouse microarray results, which showed a significant upregulation of MAP2 in the spinal cords of

Table II. Expressional alterations of microtubule-related genes in spinal cord of SMA delta 7 mice

ID	Fold Change	p-value	FDR p-value	Gene Symbol	Description
1417885_at	2.95	5.86E-06	0.0002	Mapt	microtubule-associated protein tau
*1449682_s_at	2.87	4.93E-08	5.60E-05	Tubb2a-ps2; Tubb2b	tubulin, beta 2a, pseudogene 2; tubulin, beta 2B class IIB
1424718_at	2.67	4.80E-06	0.0002	Mapt	microtubule-associated protein tau
1421327_at	2.42	0.0002	0.0019	Map2	microtubule-associated protein 2
1424719_a_at	2.41	1.13E-05	0.0003	Mapt	microtubule-associated protein tau
1450397_at	2.07	0.0039	0.0166	Map1b	microtubule-associated protein 1B
1421328_at	2.04	6.44E-05	0.0009	Map2	microtubule-associated protein 2
1425534_at	1.95	4.88E-05	0.0008	Stau2	staufen (RNA binding protein) homolog 2 (Drosophila)
1452679_at	1.88	7.85E-06	0.0003	Tubb2b	tubulin, beta 2B class IIB
1418066_at	1.79	1.01E-05	0.0003	Cfl2	cofilin 2, muscle
1428819_at	1.77	7.75E-05	0.001	Mapre1	microtubule-associated protein, RP/EB family, member 1

Table II. Continued

ID	Fold Change	p-value	FDR p-value	Gene Symbol	Description
1415978_at	1.71	0.0001	0.0016	Tubb3	tubulin, beta 3 class III
1425533_a_at	1.67	0.0002	0.0019	Stau2	staufen (RNA binding protein) homolog 2 (Drosophila)
1416256_a_at	1.59	1.44E-05	0.0004	Tubb5	tubulin, beta 5 class I
1435347_at	1.59	8.30E-05	0.0011	Stau1	staufen (RNA binding protein) homolog 1 (Drosophila)
1422765_at	1.53	0.0029	0.0133	Mapre1	microtubule-associated protein, RP/EB family, member 1
1424040_at	-1.56	0.0045	0.0186	Map7d1	MAP7 domain containing 1
1450407_a_at	-1.64	0.0012	0.0071	Anp32a	acidic (leucine-rich) nuclear phosphoprotein 32 family, member A
1418868_at	-1.67	0.0028	0.0128	En2	engrailed 2
1426518_at	-1.74	2.01E-05	0.0004	Tubgcp5	tubulin, gamma complex associated protein 5
1429894_a_at	-1.95	8.66E-05	0.0011	Map7	microtubule-associated protein 7
1423221_at	-2.08	0.0009	0.0056	Tubb4a	tubulin, beta 4A class IVA
1421836_at	-2.79	0.0001	0.0014	Map7	microtubule-associated protein 7
1421835_at	-3.21	0.0001	0.0013	Map7	microtubule-associated protein 7
1460219_at	-4.39	0.0002	0.002	Mag	myelin-associated glycoprotein

*is used where the probe group is annotated to different gene symbols

delta 7 SMA mice. We analyzed *MAP2* gene expression in the brain and spinal cord tissues of the SMA Taiwanese mouse model and detected a significant increase in *MAP2* gene expression in both tissues in the late symptomatic stage, which suggests a global differential expression of the *MAP2* gene in the central nervous system. Results obtained from earlier developmental stages of SMA mice showed that MAP2 upregulation in the spinal cord occurs during the onset of disease symptoms. MAP2 induction may be a compensatory mechanism in an impaired cytoskeletal environment to maintain both microtubule stability and actin-microtubule crosslink during disease progression. Detailed studies on MAP2 expression in both neuronal and surrounding non-neuronal cells will help to reveal any functional consequences of this alteration.

Conclusion

Our findings may indicate an altered expression of the *MAP2* gene during disease progression. Although our work is limited due to a lack of protein studies, our preliminary results indicate that microtubule regulatory proteins may be altered in SMN depleted cells. Further studies will be valuable in understanding the involvement of both MAP2 and other microtubule-related proteins to SMA patho mechanisms.

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Ethics

Ethics Committee Approval: This article does not contain any studies with human participants performed by the authors.

Informed Consent: Informed consent wasn't obtained.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Concept: G.B., H.E.Y., Design: G.B., C.S., Data Collection or Processing: G.B., C.S., N.H., Analysis or Interpretation: G.B., C.S., N.H., P.C., H.E.Y., Literature Search: G.B., C.S., Writing: G.B., C.S., N.H., P.C., H.E.Y.

Conflict of Interest: The authors have no conflicts of interest to declare.

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Case Report

A Novel Mutation in Fanconi Bickel Syndrome Diagnosed in the Neonatal Period

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ABSTRACT

Fanconi Bickel Syndrome (FBS), also known as glycogen storage disease Type XI, is a rare autosomal recessive disorder. This syndrome has many different identified mutations and it is rarely diagnosed during the neonatal period. Our patient is a two-week old female newborn who was admitted to our hospital with fever and dehydration. Renal Fanconi Syndrome was diagnosed in the presence of polyuria, proteinuria, glycosuria, hyperchloremic metabolic acidosis with normal anion gap and positive urine anion gap, hyperuricemia, hypophosphatemia and an increased excretion of phosphorus in urine. A novel mutation, IVS8 homozygote g.24401-24406del6 in the *GLUT2* gene was demonstrated by the Sanger method. The same mutation was detected as heterozygote in her parents. Although most of the affected infants have a consanguineous parentage history in the literature, our patient was born to non-consanguineous parents. Also, according to our knowledge, few FBS patients were diagnosed in the newborn period. Our patient was diagnosed with a novel mutation in her first month of life.

Keywords: Fanconi Bickel Syndrome, glycogen storage disease Type XI, mutation, neonatal period

Introduction

Fanconi Bickel Syndrome (FBS), also known as glycogen storage disease Type XI, is a rare autosomal recessive disorder. It is characterized by a mutation in the gene *GLUT2* (SLC2A2) that causes impaired glucose and galactose transportation in the kidney, intestines, liver and pancreas (1,2). Growth retardation, fasting hypoglycemia, postprandial hyperglycemia, generalized tubulopathy and hypophosphatemic rickets are the main symptoms of FBS (3). Treatment of this disorder is generally symptomatic. Here, we report an unusual patient with FBS diagnosed with a novel mutation in the *GLUT2* gene in the neonatal period.

Case Report

A two-week old female newborn was admitted to our hospital with fever and dehydration. On admission, the baby was irritable and dehydrated. Her gestation period was 38 weeks and her birth weight was 2.600 g (10-25 percentile), her height was 47 cm (10-25 percentile) and her head circumference was 35 cm (75-90 percentile) at delivery. She had non-consanguineous parents. Systemic examination was otherwise unremarkable.

Laboratory tests revealed, hyperglycemia (310 mg/dL) (normal: 60-100 mg/dL), hypouricemia (1.1 mg/dL) (normal: 2.6-6.0 mg/dL), hyponatremia (130 mmol/L) (normal:

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135-145 mmol/L), hypokalemia (3.7 mmol/L) (normal: 4.1-5.3mmol/L), hyperchloremia (121 mmol/L) (normal: 98-107 mmol/L), hypophosphatemia (2.6 mg/dL) (normal: 2.5-4.7 mg/dL), metabolic acidosis with normal anion gap (pH: 7.31 HCO3: 17 mEq/L) (normal for pH: 7.35-7.45; normal for HCO3: >15 meq/L) and elevated alkaline phosphatase (1.355 IU/L) (normal: 48-406 U/L).

Urine pH was 6.5 and urine analysis revealed proteinuria and glycosuria. Urine output was 14 cc/kg/hour. Fractional excretion of sodium was 5.8% (normal: 0.3-1.6%), tubular phosphorus reabsorption (TPR) was 65% (normal: >85%), microprotein/creatinine and microalbumin/creatinine ratios were 4.4 mg/mg Cr (normal: <0.7 mg/mg Cr) and 8.5 mg/g Cr (normal: <30 mg/g Cr) respectively. Calcium/creatinine ratio was 0.38 mg/mg Cr (normal: <0.8 mg/mg Cr). Ammonia and lactate were within normal ranges. Insulin, c-peptide and Hemoglobin A1c were 1 uU/mL (normal: 2.6-24.9 uU/mL), 0.458 ng/mL (normal: 0.9-7.1 ng/mL) and 2.5% (normal: <6%) respectively. Ophthalmologic examination was normal in terms of cystine crystals and cataract. Generalized aminoaciduria was found in urine chromatography. There was no finding associated with rickets or pathologic fracture in babygram. 25-hidroksi vitamin D3 (25-OH D3) was 7.95 ng/mL (normal: >21 ng/mL) and parathormone was 42 pg/mL (normal: 11-67 pg/mL). Renal ultrasound revealed kidneys with normal shape, size and renal cortical echogenicity. Measurement of the leukocyte cystine content with high-performance liquid chromatography was normal (0.10 nmol/protein).

Renal Fanconi syndrome was diagnosed in the presence of polyuria, proteinuria, glycosuria, hyperchloremic metabolic acidosis with normal anion gap and positive urine anion gap, hypouricemia, hypophosphatemia and increased excretion of phosphorus in the urine. Genetic analysis was performed to confirm the diagnosis. Informed consent was received from the family. A novel mutation, IVS8 homozygous g.24401-24406del6 in the *GLUT2* gene was demonstrated by the Sanger method. This mutation is seen in the C1065_1068+2delCTCTGT in the *SLC2A2* gene, according to the Human Genome Variation Society. The same mutation was detected as heterozygous in her parents by a segregation study.

Fluid-electrolyte imbalance was corrected and vitamin D, phosphorus and alkali supplementation were initiated. Ibuprofen was used for polyuria. Conservative management was used for proper maintenance of blood glucose levels and short term insulin was used when it was >300 mg/dL. Her weight reached 4.100 g in the sixth month of life, nevertheless polyuria, proteinuria, glycosuria, hyperchloremic metabolic acidosis and hypouricemia continued. She was followed-up in our out-patient clinic without any deterioration until she died in the seven month of life due to aspiration.

Discussion

FBS was first described by Guido Fanconi and Bickel (4) in 1949. It is a rare glycogen storage disease characterized by glycogen accumulation secondary to non-functional glucose transport in the liver and kidney. Severe renal tubular dysfunction and impaired glucose and galactose metabolism are the cardinal symptoms of the disease (5). The mechanism of the disease is a monosaccharide transport defect across the membranes without underlying enzymatic defect in carbohydrate metabolism (1).

FBS is caused by mutations in the *GLUT2* gene, which is located on chromosome 3. GLUT2 expressed on hepatocytes, pancreatic beta cells, basolateral membranes of intestine and renal tubular epithelial cells.

The first patient described by Fanconi and Bickel (4) in 1949 had a homozygous arg301-to-ter (R301X) mutation (5). Sakamoto et al. (6) studied three Japanese patients with FBS and found four novel mutations in the *GLUT2* gene, including a splice site mutation, a nonsense mutation, and two missense mutations. There are 14 reported mutations in Turkish patients with FBS in the literature (7). A novel mutation, IVS8 homozygous g.24401-24406del6 within the gene of the GLUT2 was detected in the genetic analysis of our patient. Although most of the affected infants in the literature have a consanguineous parentage history, our patient was born to non-consanguineous parents (8).

Our patient was diagnosed in her first month of life. According to our knowledge, few cases of FBS have been reported in the neonatal period (3). The first symptoms of this syndrome are usually recognized between 3 and 10 months of age. Diagnosis of FBS normally occurs in late infancy as FBS clinical features develop. In some cases, galactosaemia screening leads to an earlier diagnosis (3,9).

There is no specific therapy available for this syndrome. Symptomatic therapy such as replacement of water, electrolytes, vitamin D3 and phosphate and a diabetes mellitus-like diet with adequate caloric intake as frequent small meals may improve growth (10). Without oral phosphate and vitamin D supplementation, severe hypophosphatemic rickets may occur in the first months of life. After initiation of proper diet and supplements, our patient had showed partial clinical and metabolic improvement. A conservative management of hyperglycemia was carried out as recommended by Taha et al. (11) reported that the use of insulin should be avoided as it may further increase the risk of hypoglycemia, especially, in younger patients.

Our patient did not find a chance to have a longer follow-up period as she died when she was 7 months old because of a reason unrelated to her primary disease.

Our patient had a novel mutation not described in the literature so far. In addition, diagnosis in the neonatal period is a rare condition for FBS in the literature. Although

this disorder is rare, early diagnosis is important to achieve a good clinical condition and to prevent metabolic complications such as rickets by the initiation of a proper diet and supplements.

Ethics

Informed Consent: Informed consent was received from the family.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Ş.K.G., K.Ç., Ş.Ç., Concept: Ş.K.G., Ş.Ç., E.S., Design: Ş.K.G., Ş.Ç., E.S., Data Collection or Processing: Ş.K.G., K.Ç., Analysis or Interpretation: Ş.K.G., E.S., Literature Search: Ş.K.G., K.Ç., Writing: Ş.K.G., E.S.

Conflict of Interest: No conflict of interest was declared by the authors.

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Case Report

Intrapulmonary Sequestration in a 2-month-old Baby Treated Successfully by a Combination of Embolization and Surgical Resection

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ABSTRACT

Intralobar pulmonary sequestration is a rare congenital malformation, usually diagnosed later in childhood or adolescence. We report a case who presented with tachypnea and was diagnosed at 2 months of age. Pulmonary sequestration is usually managed by embolization or surgical resection. Recently, preoperative embolization of aberrant arteries to minimize the risk of serious intraoperative hemorrhage has also been described. Our case was successfully treated with embolization followed by a thoracoscopic resection.

Keywords: Embolization, pulmonary sequestration, surgical resection

Introduction

Pulmonary sequestration (PS) represents 0.15-6.4% of all congenital pulmonary malformations (1). The 2 forms of PS are intralobar pulmonary sequestration (ILS), which is surrounded by normal lung tissue, and extralobar (ELS), which has its own pleural investment. Although many patients with ELS present with respiratory distress and chronic cough in infancy, ILS is usually diagnosed later in childhood or adolescence. However, occasionally, symptoms may begin early in childhood in patients with ILS. Recurrent pneumonia, chronic or recurrent cough or hemoptysis are the most common presenting symptoms. Patients with PS may have a systolic bruit or continuous murmur over the affected area. This is related to blood flow through the sequestration from the large systemic arterial supply. Diagnosing ILS is based on imaging and identifying the systemic arterial supply. The classical treatment in PS is surgical resection or embolization. Preoperative embolization of feeding arteries followed by surgical resection is a new treatment option for those patients with a high risk of intraoperative hemorrhage.

Here, we present a 2-month-old boy who was diagnosed as ILS and on whom embolization was performed firstly to allow a safe surgical resection.

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Case Report

This case study concerns a 2-month-old boy, who was born at normal gestation after a normal pregnancy. His birth weight was 3.500 gm and Apgar scores were 8 and 10 at 1 and 5 minutes, respectively. At 2 months of age, he was admitted to the Pediatric Pulmonology, Ege University Medical Faculty because of tachypnea. Physical examination revealed tachypnea (68/minute), subcostal retractions and 3/6 pansystolic murmur over the affected area. Laboratory examination revealed a white blood cell count of 12.100/µL (normal range: 6.000-17.500); hemoglobin: 11.6 g/dL (normal range: 10.3-14.1), a serum C-reactive protein of 0.1 mg/dL (normal range: 0-0.5) and arterial blood gases as normal. Echocardiography was normal. Chest radiography showed a non-specific hazy opacification in the left lung (Figure 1). Thoracic ultrasound confirmed a heterogenous, tubular, 2.5 cm by 1.5 cm smooth well-defined lesion (Figure 2). computed tomography (CT) of the chest and CT angiography showed a consolidation (largest diameter 2.5 cm by 1.5 cm) in the posterior segment of the inferior left lobe and feeding arteries taking off from the distal thoracic aorta which was reported as an ILS (Figure 3A, B). The diagnosis was ILS located at the posterior segment in the left inferior lobe. His prenatal ultrasound was normal. Given the history, imaging features characteristic of ILS and ongoing symptoms, the patient was referred for surgery. Our patient had a continuous murmur over the affected area which was related to blood flow through the sequestration from the large systemic arterial supply. We performed preoperative embolization of aberrant arteries in order to minimize the risk of serious intraoperative hemorrhage (Figure 4A, B). Thoracoscopic lobectomy was performed 7 days after the embolization (Figure 5).

Histopathological examination of resected material showed malformed multiple vascular structures of

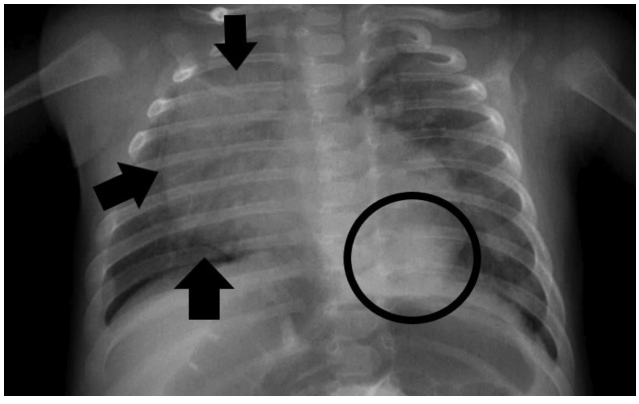

Figure 1. Chest radiograph at the time of first admission shows rounded density in the left lower lobe. Thymus gland is taking place in the right superior and midlle zone (shown with arrows)

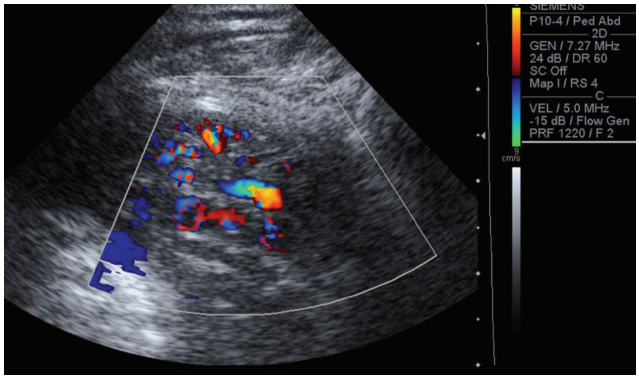

Figure 2. Thoracic ultrasonography shows large diameter arteriovenouse fistulisation that is placed in the pulmonary sequestration

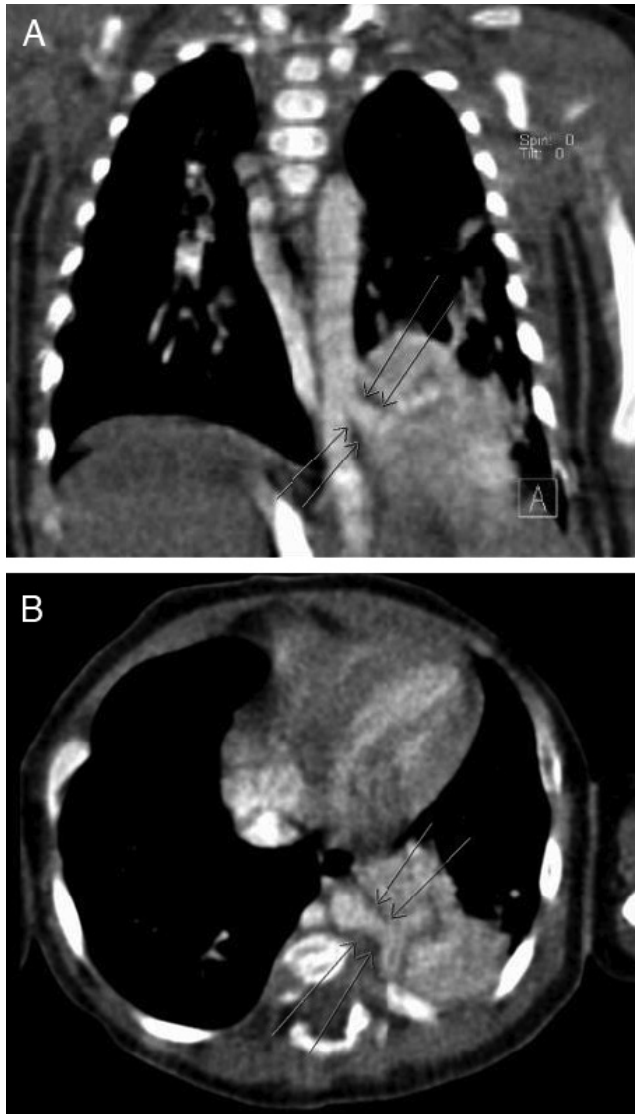

Figure 3. A) Computed tomography-pulmonary angiogram showing the feeding artery emanating from the aorta and supplying blood to the sequestration B) Computed tomography-pulmonary angiogram showing the feeding artery emanating from the aorta and supplying blood to the sequestration

different sizes with a thickened wall showing intimal proliferations and immature, atelectatic lung parenchyma (Figure 6).

The patient postoperative course was uncomplicated. He was hospitalized for 10 days after the surgery and discharged without any complication. No further complications were observed during the post-operation follow-up of 6 months. Informed consent was obtained from our patients' parents.

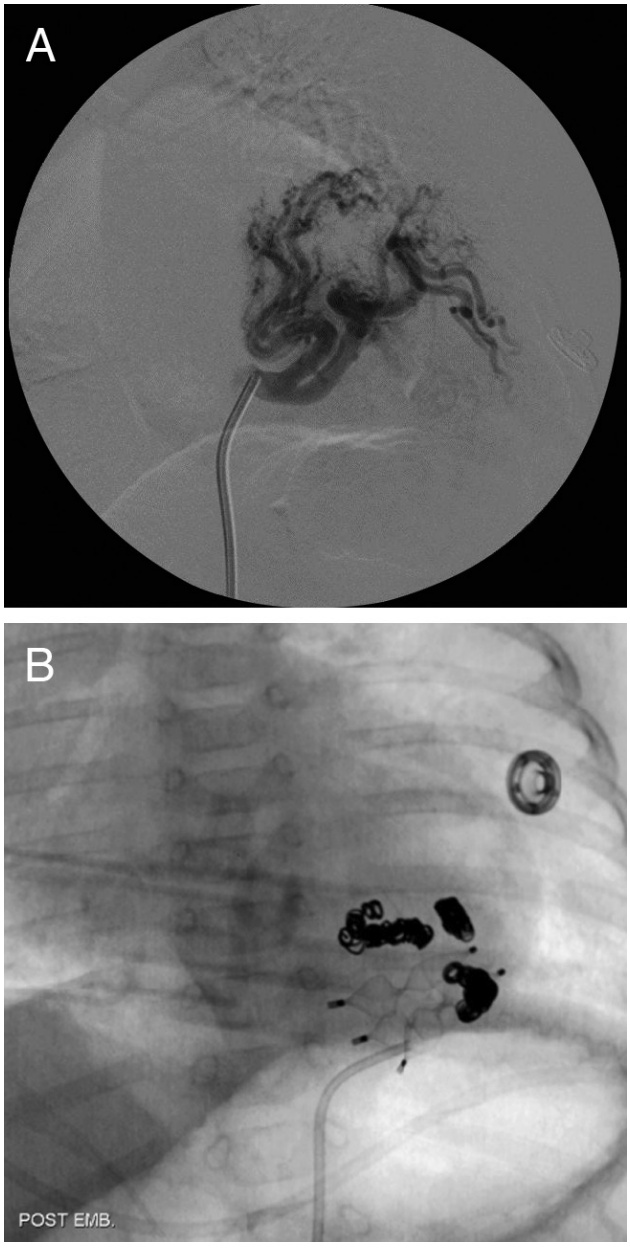

Figure 4. A) Coil embolization is performed to minimize bleeding during thoracoscopic lobectomy B) Coil embolization is performed to minimize bleeding during thoracoscopic lobectomy

Discussion

Pulmonary sequestration is a rare congenital anomaly of the lungs characterized by non-functional, dysplastic lung tissue which does not have a connection with the tracheobronchial tree (2). This tissue is usually supplied by branches of abnormal systemic arteries which are generally from the thoracic aorta (46.1-86.1%) and occasionally from the abdominal aorta (6.9-31.6%). The other feeding supplies can include the intercostal artery, diaphragmatic artery, aortic arch, subclavian artery, pulmonary artery, left gastric artery, coronary artery, arteria lienalis, celiac truncus and renal artery (3).

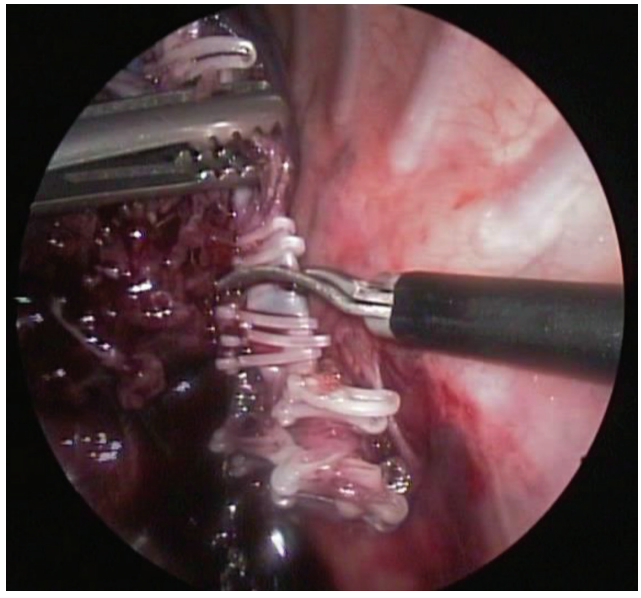

Figure 5. Left lower lobe was resected after coil embolization had been completed

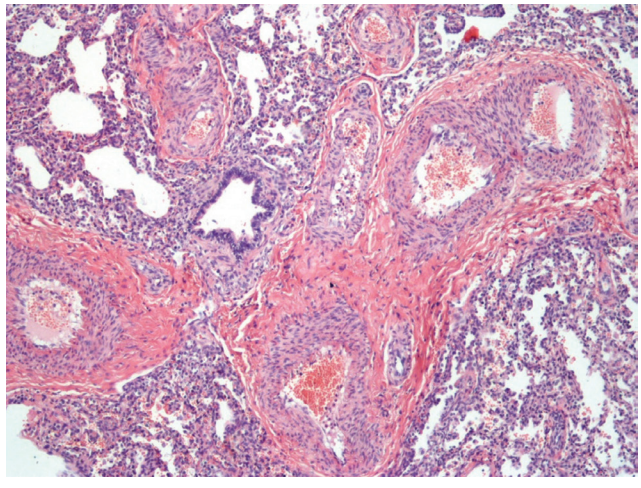

Figure 6. Thick walled, variable sized, numerous malformed vascular structures and immature lung parenchyma (Hematoxylin and eosin stain, x4)

Although prenatal ultrasound plays an important role in the diagnosis of PS, 84.3% of patients diagnosed during the prenatal period via our patient prenatal ultrasound did not have the diagnosis confirmed (4). Ultrasound has limited value for the follow up after the prenatal period. PS also can be identified by contrast-enhanced CT. However, CT-pulmonary angiography is the best method for identifying the arterial supply (5). In our patient, CT-pulmonary angiography demonstrated that there were at least two feeding vessels branching from the thoracic aorta going directly into the ILS. PS is divided into two categories; ILS and ELS. ILS occurs within the visceral pleura of normal lung tissue. The most common location is in the left lower lobe. ELS is completely enclosed in its own pleural sac and nearly all are also located on the left side (6). Many patients with ELS are diagnosed in the prenatal period by polyhydramnios or hydrops. ILS is usually diagnosed later in childhood or commonly after the second decade (7). Occasionally, respiratory symptoms may begin early in childhood as in our case. The location of our patient's lesion in the posterior segment of the inferior left lobe was characteristic for ILS. He presented with tachypnea at 2 months old contrary to most patients who are diagnosed later in life. Our patient's physical examination revealed pansystolic murmur over the affected lung area which was related to a large left to right shunt, which can cause congestive heart failure in undiagnosed and untreated patients. Patients with ILS often require surgery or embolization which are both effective and safe treatments for PS (8). However, the ideal treatment strategy for PS, either resection or embolization in childhood, is not clearly defined. Surgical resection is preferred in patients with large caliber shunts, giant lesions covering an entire lobe, renal failure, secondary pulmonary hypertension or an unsuccessful embolization (9). However, potentially life threatening hemorrhage during pulmonary resection is one of most important complications especially in those patients with large feeding arteries as in our case. In order to overcome this problem, preoperative embolization followed by surgical resection has recently been used (10,11). Since our patient had a large systemic arterial supply, we performed preoperative embolization of aberrant arteries in order to minimize the risk of serious intraoperative hemorrhage. Histopathological examination of our patient's resected material showed malformed multiple vascular structures of different sizes with thickened walls showing intimal proliferations and immature atelectatic lung parenchyma which is compatible to ILS. In pathological specimens of PS, there are prominent vascular lumina within the sequestered parenchyma, with markedly thickened arteries and focal subendothelial fibro intimal proliferation as well as moderate focal thickening of the muscularis and adventitia. The parenchyma is structurally abnormal with thickened airspace walls, poorly subdivided airspaces and large dilated airways (12).

In conclusion, we reported a 2-month-old boy with ILS who presented with tachypnea. Tachypnea is a very common presenting symptom during infancy for a variety of diseases including pulmonary, cardiac, metabolic or intracranial. However, in our case, consolidation in his chest X-ray and systolic murmur over the affected area led us to proceed to further investigations and finally to a diagnosis of ILS. Currently available treatment strategies for PS are embolization and surgical resection. In our case, we successfully combined these two treatment modalities in order to prevent intraoperative hemorrhage since our patient had large caliber feeding vessels.

Ethics

Informed Consent: Informed consent was obtained from our patients' parents.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: C.Ç., H.A.E., E.D., Concept: A.E., E.D., Design: E.D., F.G., Data Collection or Processing: A.E., G.K.Ö., Analysis or Interpretation: E.D., T.M., H.H.A., Literature Search: A.E., G.K.Ö., T.M., C.Ç., D.N., Writing: A.E., E.D., F.G.

Conflict of Interest: No conflict of interest was declared by the authors.

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Case Report

Hereditary Neuropathy with Liability to Pressure Palsy: A Case Diagnosed with a Quick Multiplex Ligation-dependent Probe Amplification Test

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ABSTRACT

Hereditary neuropathy with a liability to pressure palsies (HNPP) represented by recurrent focal pressure neuropathies is rare in childhood. Here we present a 10-year-old girl admitted to our hospital with a recurrent weakness in her foot and diagnosed as HNPP with a quick Multiplex Ligation-dependent Probe Amplification test revealing PMP22 deletion.

Keywords: Hereditary neuropathy, pressure neuropathy, mjultiplex ligation-dependent probe amplification

Introduction

Hereditary neuropathy with a liability to pressure palsy (HNPP) is known as an autosomal dominant inherited neuropathy (1). It presents with recurrent sensory and motor nerve palsies usually caused by compression or minor trauma. Although HNPP is rarely reported in childhood, it is probably under-diagnosed due to its wide spectrum of clinical manifestations. Early diagnosis is important to provide appropriate genetic counseling to families, to provide appropriate care for these patients, and to prevent unnecessary investigations (2).

HNPP is diagnosed by genetic tests revealing 90% of cases, including the *PMP22* gene, of a 1.5 Mb chromosome 17p11.2 deletion. However, duplications involving the same gene cause a distinct genetic condition, namely CMT1A, which is the most common type representing approximately 70 to 80% of all CMTs. HNPP also results from *PMP22* gene mutations that alter a single amino acid in the PMP22 protein or that lead to the production of an abnormally small protein.

The incidence of CMT1A and HNPP is as high as 1 in every 2.500 persons (3). Electrophysiological studies are important for differential diagnosis to verify the presence of focal abnormalities in HNPP and to guide genetic studies by revealing an underlying demyelinating polyneuropathy (4).

Real-time quantitative polymerase chain reaction is also very sensitive for identifying the *PMP22* gene copy number in CMT1A duplication and HNPP deletion (5). The deletion is usually detected by fluorescence *in situ* hybridisation (FISH). However, this approach is time-consuming and cannot detect small intragenic rearrangements. On the other hand,

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whole-exome sequencing (WES) is not recommended as the first step since the costs are still high and the sensitivity of this technology is not yet high enough (6).

Recently, Multiplex Ligation-dependent Probe Amplification (MLPA) assays have been proposed as a fast, simple and cost-effective technique for the molecular diagnosis of CMT1A and HNPP (7). Here we report a case with HNPP diagnosed with a quick MLPA test.

Case Report

A 10-year-old girl was admitted with a sudden onset of weakness that started on her left foot fifteen days previously. There was no pain and symptoms were unresponsive to oral methylprednisolone treatment started in the regional hospital with a diagnosis of polyneuropathy one week previously. Her past medical history was not significant with respect to trauma, toxic exposure, injection or infection. However, one year previously she had a similar symptom in her right foot which developed after sitting on her right leg that resolved itself spontaneously within a week. At that time, she was evaluated at another hospital and her cranial magnetic resonance imaging (MRI) was normal. Her family history was unremarkable.

Neurological examination revealed a loss of dorsiflexion ability in her left foot. Steppage gait was present. Bilateral patellar and achilles reflexes were hypoactive. Complete blood count, biochemistry, lipid profile, acute phase reactants, vitamin B_{12}, E, A levels, laboratory tests for vasculitic disorders were normal. Cranial and spinal MRI were also normal.

Electromyography (EMG) revealed electrophysiological findings of bilateral carpal tunnel syndrome, cubital tunnel syndrome and fibular nerve neuropathy. With this history, neurological examination and EMG findings, HNPP was considered as a preliminary diagnosis.

For definite diagnosis, deletion and duplication studies were performed with 9 (nine) probes specific for 5 (five) exons of the *PMP22* gene located in the 17p12 region, TEXT3 (exons 3 and 9) and *COX10* (exon 7) genes located in the close vicinity of PMP22 by MLPA method. Heterozygous deletions including all exons of *PMP22* plus *TEKT3* (exons 3 and 9) and *COX10* (exon 7) genes were detected (Figure 1). DNA microarray analysis performed to detect deletion borders revealed a deletion of 1.360 Mb including *HS3ST3B1, PMP22* and *TEKT3* genes in the 17p12 region.

An ankle-foot orthosis was applied to alleviate right foot drop. Full recovery was observed within one month. Protective pads for elbows or knees were recommended to prevent pressure and trauma to local nerves. The patient was advised to avoid sitting with her legs crossed, leaning on elbows for long periods, repetitive movement of the wrists and rapid weight loss. Written informed consent was obtained from the family for the publication of this case report.

Discussion

HNPP is characterized by recurring focal pressure neuropathies such as peroneal palsy with foot drop and carpal tunnel syndrome. HNPP is underdiagnosed due to phenotypic heterogeneity. Family history should be carefully reviewed to identify undiagnosed potential HNPP cases (2). The presented case did not have a family history of HNPP and was diagnosed with a quick MLPA test after the initial electrophysiological evaluation.

The clinical spectrum of HNPP ranges from mononeuropathies to recurrent episodes of brachial

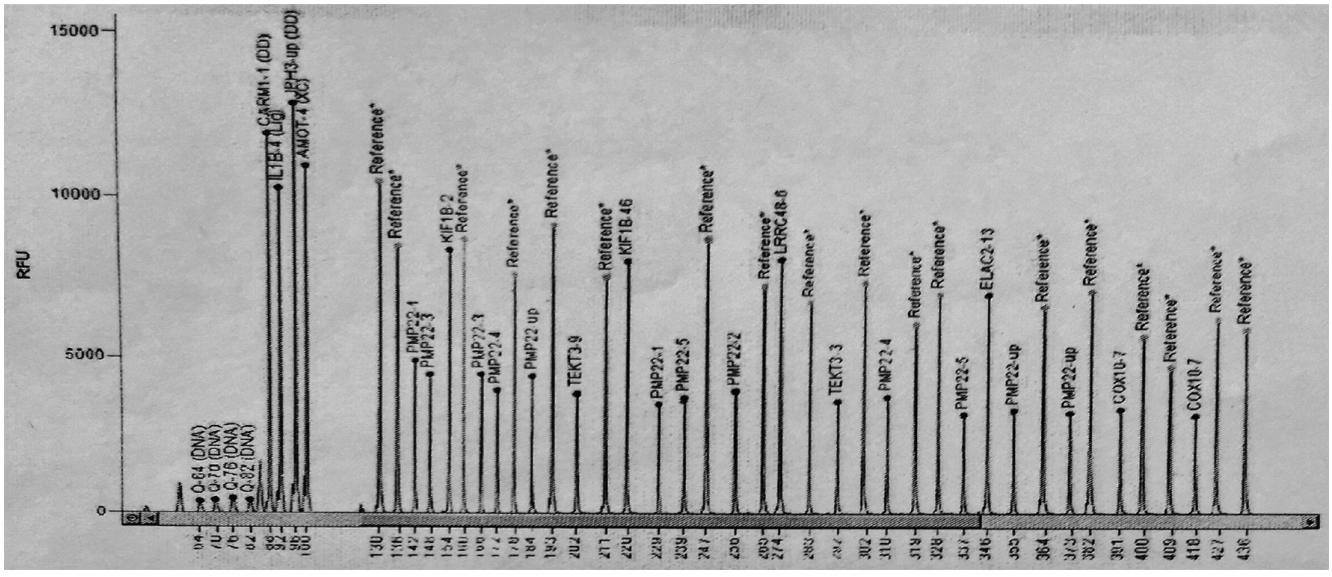

Figure 1. Heterozygous deletions including all exons of PMP22, plus TEKT3 and COX10

plexopathy. Reportedly, peroneal palsy is the most common presentation (42%) followed by brachial plexus palsy (2). The most common findings related with HNPP are the existence of polyneuropathy, median terminal motor latency prolongation and multiple compression neuropathies. In our case, there was a weakness only in the left foot while having electrophysiological findings of bilateral carpal tunnel syndrome, cubital tunnel syndrome and fibular nerve neuropathy.

The clinical suspicion of HNPP should be referred to genetic testing, even when study results of nerve conduction do not meet HNPP criteria. Light microscopic changes in nerve biopsy are not specific for this disease. Genetic testing is the first choice because it is non-invasive. PMP22 is the only gene that has been shown to be associated with HNPP. An adjacent gene deletion of chromosome 17p11.2 containing PMP22 is found in about 85% of the affected individuals, while the remaining 25% have a pathogenic variant of PMP22 (7).

Recently, a number of tools have been developed for the detection of copy number variations based on new generation sequencing data. WES was recommended in patients diagnosed with CMT1A or HNPP using STR markers to assess the ability of WES to improve the clinical diagnosis. However, use of these methods is limited (4). Due to a large number of genes that can be analyzed by a single technique, the MLPA test represents the gold standard for the molecular analysis of all pathologies derived from the presence of gene copy number variation.

Slater et al. (3) investigated the utility of the MLPA assay in the detection of PMP22 duplications and deletions for the molecular diagnosis of CMT1A and HNPP. The performance of MLPA is compared to one of the interphase FISH analyzes. MLPA assays represent a robust, simple and cost-effective approach for the molecular diagnosis of CMT1A and HNPP (3). The presented case provides additional support to the utility of MLPA assays in the rapid detection of PMP22 duplications and deletions for the molecular diagnosis of patients with HNPP.

In conclusion, HNPP should be considered in recurrent, episodic, painless and entrapment neuropathies after exposure to pressure or trauma. As a result of early diagnosis, high-cost, invasive tests and unnecessary treatments for the prognosis of the disease can be avoided.

Ethics

Informed Consent: Written informed consent was obtained from the family for the publication of this case report.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: S.K., E.Ş., H.M.S., M.K.E., S.Y., G.A., H.T., S.G., Concept: S.K., H.T., Design: S.K., E.Ş., H.M.S., H.T., Data Collection or Processing: S.K., E.Ş., H.M.S., M.K.E., Analysis or Interpretation: H.M.S., S.Y., H.T., Literature Search: S.K., Writing: S.K.

Conflict of Interest: No conflict of interest was declared by the authors.

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Case Report

Plastic Bronchitis Following Fontan Procedure: A Case Report

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ABSTRACT

Fibrinous bronchitis, also known as plastic bronchitis or pseudomembranous bronchitis, usually manifests clinically with non-productive cough and dyspnea. Prior to the expectoration of plaque formations, patients have a history of viral or other respiratory disease. Plastic bronchitis is an extremely rare and fatal complication associated with Fontan procedure. The condition is characterized by the formation of inspissated bronchial casts which may cause life-threatening airway obstructions. Although the pathogenesis of this condition remains unclear, it is believed to involve elevated pulmonary venous pressure, increased central venous pressure, and endobronchial lymphatic leakage. A 9-year-old male patient with Down syndrome underwent Fontan procedure 29 months earlier due to complete endocardial cushion defect and single ventricular physiology. The patient presented to the emergency department due to sudden-onset respiratory distress and fever (38.5 °C). Bilateral diffuse sibilant rhonchi, secretory rales, and intercostal retractions were noted during pulmonary system examination. Posterior-anterior chest x-ray showed cardiomegaly (cardiothoracic ratio=0.6) and bilateral diffuse infiltration. While under treatment, the patient experienced sudden-onset cough with expectoration of rubbery sputum in the form of branching bronchi-shaped casts. Samples of the inspissated sputum were sent to the Pathology and Microbiology departments for examination. The pathology report indicated fibrinoid material composed of a small number of inflammatory cells and bacterial plaques. Normal bacterial flora was identified in microbiological culture. Clinical presentation can vary from mild clinical findings to life-threatening symptoms. As in our patient, the diagnosis is made clinically, based on expectoration of bronchial casts or their detection during bronchoscopy. As our patient responded well to medical treatment, we proceeded with clinical follow-up. Plastic bronchitis is a very rare entity, and carries a poorer prognosis when it develops after congenital heart disease, as in our case.

Keywords: Plastic bronchitis, fontan operation, fibrinous bronchitis

Introduction

Fibrinous bronchitis, also known as plastic bronchitis or pseudomembranous bronchitis, usually manifests clinically with non-productive cough and dyspnea. Prior to the expectoration of plaque formations, patients have a history of viral or other respiratory disease (1-3). Plastic bronchitis is an extremely rare and fatal complication associated with Fontan procedure. The condition is characterized by the formation of inspissated bronchial casts which may cause life-threatening airway obstruction. Although the pathogenesis of this condition remains unclear, it is believed to involve elevated pulmonary venous pressure, increased central venous pressure, and endobronchial lymphatic leakage (4-6).

Case Report

A 9-year-old male patient with Down syndrome underwent Fontan procedure 29 months earlier due to complete endocardial cushion defect and single ventricular physiology. The patient presented to the emergency

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department due to sudden-onset respiratory distress and fever (38.5 °C). Bilateral diffuse sibilant rhonchi, secretory rales, and intercostal retractions were noted during pulmonary system examination. Posterior-anterior chest x-ray showed cardiomegaly (cardiothoracic ratio=0.6) and bilateral diffuse infiltration (Figure 1).

In biochemical analysis, leukocyte count was $5.53x10^3/\mu L$, absolute neutrophil count was $3.4x10^3/\mu L$, and C-reactive protein level was 0.75 mg/dL. Treatment was initiated with ceftriaxone 100 mg/kg twice daily, salbutamol 0.15 mg/kg four times daily, and fluticasone 250 mcg twice daily. Transthoracic echocardiography revealed the Fontan procedure, large atrial and ventricular septal defects, no pericardial effusion, maximum 80 mmHg gradient in the area of pulmonary artery banding, and first-degree atrioventricular valve insufficiency. While under treatment, the patient experienced sudden-onset cough with expectoration of rubbery sputum in the form of branching bronchi-shaped casts (Figure 2).

Samples of the inspissated sputum were sent to the Pathology and Microbiology departments for examination. The pathology report indicated fibrinoid material composed of a small number of inflammatory cells and bacterial plaques. Normal bacterial flora was identified in microbiological culture. In bronchoscopy performed after 7 days of antibiotic therapy, the trachea, main carina, and right and left bronchial systems appeared normal. The absence of any signs of pathology was attributed to the period of antibiotic and inhalation treatment prior to performing the bronchoscopy. The patient's lung auscultation findings resolved during follow-up. Informed consent was obtained. Antibiotic therapy was completed within 14 days and the patient was discharged with instructions to continue nebulizer treatment at home.

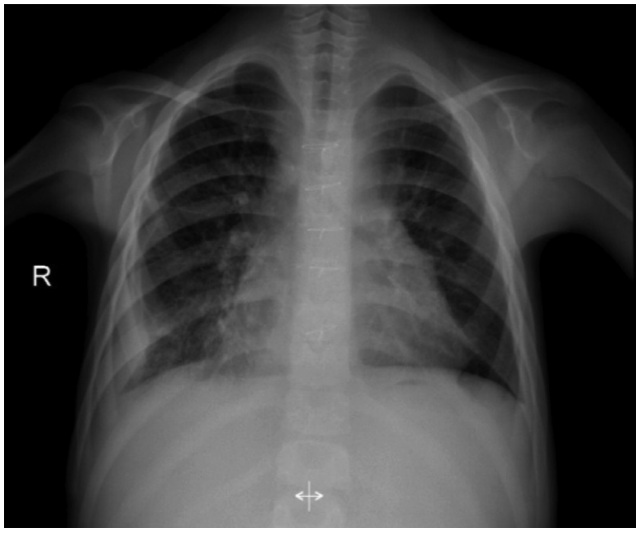

Figure 1. Bilateral diffuse infiltration and increased cardiothoracic ratio on posterior-anterior chest X-ray

Discussion

Plastic bronchitis was first reported in 131-200 AD by Galen, who described the condition as the expectoration of arteries and veins. Characterized by pathognomonic bronchial casts, the disease was previously described as fibrinous bronchitis and pseudomembranous bronchitis, but has become known as plastic bronchitis in the 20th century (7). Although plastic bronchitis can occur in all age groups, it is more common in childhood, particularly in children over 6 years old. The sex ratio among patients is generally balanced, though some studies have reported female predominance (7,8).

The pathogenesis of plastic bronchitis is not fully understood. Various mechanisms have been proposed, including elevated pulmonary venous pressure, increased inflammatory response, trauma to the bronchial lymphatic system, and ischemia of the bronchial tree (4-6).

Clinical presentation can vary from mild clinical findings to life-threatening symptoms. As in our patient, the diagnosis is made clinically, based on expectoration of bronchial casts or their detection during bronchoscopy. Patients generally present with wheezing, chest pain, fever, and coughing. Atelectasia and infiltration are usually seen in radiological imaging. Computed tomography can be effective in visualizing affected major airways (9).

Seear et al. (10) proposed a classification of two groups based on histological patterns. In Type I, the bronchial casts are composed of fibrin and dense eosinophilic infiltrate

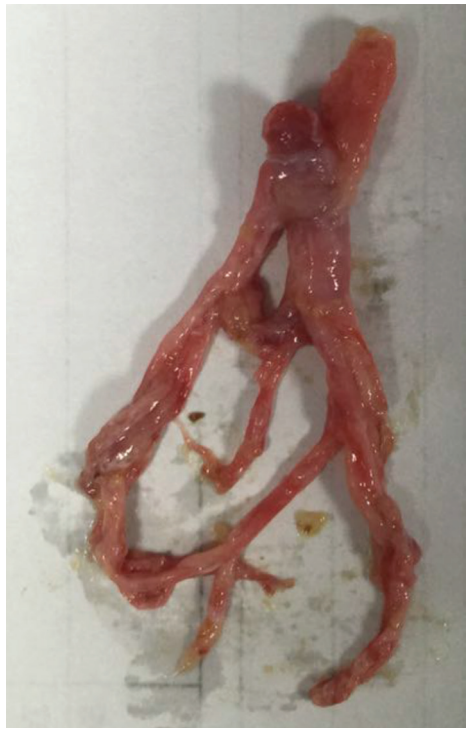

Figure 2. Mucoid bronchial casts expectorated by the patient

and occur due to acute bronchopulmonary events, while Type II casts contain mucin and are chronic and recurrent in patients who have undergone surgery for congenital cyanotic heart disease. Our patient had Type II casts, which placed him at high risk for mortality.

Treatment strategies vary from medical treatment including steroids and various inhaled lytic agents, to bronchoscopy and other surgical interventions. Although corticosteroids are mostly used in the Type I patient group, they can be used in Type II patients as well. Treatment with various medications such as tissue plasminogen activators, acetylcysteine, macrolide antibiotics, urokinase, and DNase has been attempted via nebulized delivery. Bronchoscopy is routinely practiced in both groups and can be used repeatedly to clear the large airways (11-15).

Some authors have reported performing thoracic duct ligation as treatment. In one such report, high intrathoracic lymphatic pressure was believed to be the cause of recurrent, medically refractory cast formation in two patients who developed plastic bronchitis after undergoing Fontan operations. The patients were reported to be asymptomatic for two years following thoracic duct ligation. The authors stated that thoracic duct ligation cured patients by decreasing intrathoracic lymphatic flow and pressure. However, these two patients were classified as Type II, and it has been suggested that this approach cannot be used for Type I plastic bronchitis (16,17). As our patient responded well to medical treatment, we proceeded with clinical follow-up.

Plastic bronchitis is a very rare entity, and carries a poorer prognosis when it develops after congenital heart disease, as in our case.

Ethics

Informed Consent: Informed consent was obtained.
Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: E.D., Z.Ü., Concept: E.D., E.L., Design: E.D., Data Collection or Processing: Z.Ü., Analysis or Interpretation: E.D., E.L., Literature Search: D.A., E.L., Writing: E.D., D.A.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: There are no financial conflicts of interest to disclose.

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